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**REPORT OF THE
NORTHERN REGIONAL RESEARCH CENTER**

May 1983

PRODUCTION SECTION
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PROGRESS REPORT
OF THE
NORTHERN REGIONAL RESEARCH CENTER
MAY 1983

INTRODUCTION

The Northern Regional Research Center is one of the major research facilities of the Agricultural Research Service, U.S. Department of Agriculture. The Center gives emphasis to post-harvest research to increase the use and cash value of farm products and by-products as well as to decrease losses during storage, processing, and transport due to intrinsic deterioration or external agents such as insects and microorganisms.

The resources of the Center are focused on three goals of importance to agriculture and the consumer:

- Ensured quality and safety of food for domestic and export markets
- Reduced dependence on nonrenewable resources
- Increased efficiency of plant production and protection

To meet these goals, research is organized to create knowledge or technology within the following programs:

- Protection of food from toxins
- The basis for reducing post-harvest losses
- Relationship between plant constituents and their functionality or nutritive value
- Microbial systematics and genetics
- Biotechnology
- Control of plant-pest interaction
- Regulation of biosynthesis in plants
- Beneficial plant-microbe associations

The fields of science undergirding these programs include microbial germplasm and ecology, regulation of plant and microbial metabolism, gene transfer and expression, chemistry and physics of natural polymers, and the molecular basis of functionality.

Much of the research involves crops of major importance to the north central region of the U.S., although plants of potential importance as a source of strategic materials or biomass are included.

The Center is organized into five Research Laboratories, each constituted of approximately 30 scientists plus technical support personnel. While some research programs are the exclusive province of a single Laboratory, others involve the participation by scientists in several Laboratories through formal and informal cooperation. The Northern Agricultural Energy Center is part of the Northern Center. Its studies on fermentation and chemical processes are done here while agronomic aspects are done elsewhere under cooperative agreements. The research is coordinated with that of its counterpart at Tifton, GA.

Scientists at the Northern Center cooperate with scientists at other ARS facilities, universities, state experiment stations, research institutions, industrial organizations, and other government agencies. Much of the cooperation is informal, but some work is done under cooperative agreements and memoranda of understanding. In addition, the research is supplemented by projects in foreign countries under the aegis of Public Law 480.

This report summarizes research done at the Center during 1982 and lists also the resulting publications and patents. The summaries include some preliminary results that have not been tested sufficiently to justify general release. Because of this, the report is not intended for publication and should not be referenced in literature citations. When adequately confirmed, such results will be released promptly through standard publication channels.

SELECTED ACCOMPLISHMENTS

Improved Pretreatment of Lignocellulose. Current techniques used to disrupt lignocellulosic materials and increase the accessibility of the cellulose to enzymes or chemicals often result in low conversion of the polysaccharide to glucose. Studies with model oxidants as delignifying agents have shown that H_2O_2 under certain conditions is effective in delignifying wood, straw, and corn stalks. Treatment of the lignocellulosics with H_2O_2 at pH >10 and at room temperature produces a cellulose-containing residue that shows greater than 90% conversion of the cellulose to glucose with cellulase from Trichoderma reesei. The glucose formed is fermented to ethanol with high efficiency. [See Biomaterials Conversion Laboratory (BC), C.1.]

New Technique Studies Lignin in situ. Current knowledge on lignin structure is based on modified lignin, since it is not possible to remove lignin from plants without partially degrading and modifying its structure. In order to develop more insight into the role that lignin performs in plant structural tissues and new concepts for delignifying these tissues, methods are needed that allow study of the lignin polymer as it exists in situ. Photo-acoustic-spectroscopy (PAS) has proven to be such a method. It is a nondestructive analytical technique that provides ultraviolet, visible, and infrared absorption spectra of lignin in intact plant tissues. We have now constructed a complete, dual channel UV-visible PAS system and have utilized it to characterize the light-absorption properties of in situ lignin in wheat straw and a variety of woods. It was discovered that high molecular weight lignin in situ is often significantly modified, apparently as the result of photochemical oxidation, into more highly conjugated forms which absorb light in the visible region, giving these materials their characteristic yellowish colors. Model reactions using thermomechanical pulps and newsprint indicated that near UV light absorption by lignin, and the subsequent generation of singlet oxygen, were primarily responsible for these chemical modifications. (See BC, C.1.)

New Analyses for Guayule. To enhance establishment of guayule as a domestic source of rubber, plant breeders, and other scientists have need for a standardized, rapid, accurate, and relatively inexpensive method for determining the rubber and resin content in guayule. A cooperative effort between scientists at NRRC and the ARS Water Conservation Laboratory, Phoenix, AZ, has resulted in development of a gravimetric method based on two 30-second extractions (one for resin and one for rubber) of the shrub in a high-intensity homogenizer-grinder. The two components are completely removed during the short extraction and there is not cross contamination in the extracts. Comparison of extraction data with the standard soxhlet extraction procedure reveals that degradation of high-molecular-weight rubber occurs during the lengthy extraction periods specified in the currently used standard method. (See BC, C.5.)

Alpha-Amylase Hydrolysis Rates of Corn Starches Related to Granule Size and Amylose Content. Ordinary corn starch characteristics (amylose content, gelatinization temperature, enzyme digestibility) are relatively independent

of granule size. This is not true with high-amylose starches. This work has shown that amylose content and gelatinization temperature of these starches vary inversely with granule size. Hydrolysis rate with α -amylase is only partially predictable, because the smallest granules, with the greatest surface area, also contain the most amylose, which increases resistance to amylase attack. Because high-amylose starches are very heterogeneous in granule size, these variations in properties are quite important in the processing and end use of these starches. [See Cereal Science and Foods Laboratory (CSF), A.1.]

High-Performance Reverse Phase Liquid Chromatography of Cereal Proteins. Proteins of cereal grains have been separated by reverse phase high performance liquid chromatography (RP-HPLC) for the first time. In comparison with currently used methods of electrophoresis, RP-HPLC is more rapid (1 hour), more sensitive, more reproducible, easier to carry out, and lends itself more readily to automation and computer assisted analysis and quantitation. The analysis of protein extracts from wheat or corn results in chromatographic separation patterns that are characteristic of the variety or inbred. The method can therefore be used to identify grain genotypes and to follow changes in proteins and linked genetic characteristics during breeding programs. Since the method depends on hydrophobic bonding of the proteins to highly porous chromatographic columns, the separation obtained differs from existing procedures and therefore augments information provided by them. It also can be scaled up for preparative isolations of purified proteins. (See CSF, A.2.)

Cellulose Microfibrils for Improvement of Alcohol Production. A method has been devised to routinely obtain an isolate from commercial preparation of the cellulase enzyme complex elaborated by *Trichoderma reesei* that causes filter paper to appear to swell and fracture into short fibers with the simultaneous generation of microfibrils. This action occurs without significant hydrolysis of the substrate. Partial characterization of the isolate indicates it consists of a low molecular weight factor (or factors) that is (are) nonenzymatic. The chemical characterization of this isolate could reveal the means to increase the effectiveness of cellulase systems to degrade cellulose into sugars for alcohol fermentation. (See CSF, B.1.)

Human Absorption and Retention of Zinc from Endosperms in Corn Food Products. Cooperative studies with the ARS Human Nutrition Research Laboratory, Grand Forks, ND, involving prepared endosperm food products intrinsically labelled with zinc-65 and included in special meals, established the absorption of zinc by humans at 44% and 41% for corn grits and corn flakes, respectively. Zinc-65 absorption and retention was measured by total body counting techniques. However, for the intrinsically labeled corn flakes, where the formation of Maillard products had occurred, the retention time of the zinc absorbed by humans was shorter than for the nontosted corn grits. (See CSF, C.1.)

A Better Method for Obtaining Labeled Phytic Acid Has Been Developed. Use of germinating wheat seeds to produce labeled phytic acid can now be done

reproducibly to obtain either a tritium phosphorus, or ^{14}C label or any combination of these attached onto the phytic acid molecule. Minimum plant residues result from this technique which allows one to prepare labeled material at relatively high specific activity. The ability to prepare such labeled materials means that fundamental studies such as equilibrium dialysis studies of mineral binding with proteins and phytate can now be carried out to investigate interactions of phytates with foods and minerals. (See CSF, D.1.)

New Lectin Activity in Soybeans. A hitherto unrecognized carbohydrate-binding protein has been found in seeds of all soybean varieties examined, including both "lectinless" and non-nodulating ones. In contrast to the classic, D-galactose-specific soybean hemagglutinin, the new lectin has multivalent binding specificity toward 4-O-methyl-D-glucuronic acid, which is the side-chain component of the extracellular polysaccharide formed by many strains of Rhizobium japonicum. The receptor is also present on the surfaces of washed cells. This finding lends new credence to the notion that initial recognition leading to establishment of the symbiosis is between plant lectin and microbial surface polysaccharide. It also explains why genetically lectinless varieties lacking the hemagglutinin are readily nodulated in the field [See Fermentation Laboratory (F), A.1.]

Rapid Utilization by Soils of Nitrogen Stored in Blue-Green Algae. A significant fraction of nitrogen in blue-green algae is in the form of insoluble granules that consist of an arginine-aspartic acid (1:1) polypeptide called cyanophycin. The granules are laid down in senescent cells during development of sporelike forms (akinetes). Because Azolla plants contain large amounts of cyanophycin, a high proportion of their nitrogen-fixing blue-green algal symbionts appear to be senescent. A surprising finding has been the conversion of radiolabeled cyanophycin to CO_2 by paddy and other soils. This conversion proceeds at a rate comparable to that for other forms of algal nitrogen even though the cyanophycin is resistant to known proteolytic enzymes. These results shed new light on the agronomic contribution of blue-green algae and Azolla. (See F, A.2.)

Relationships of Microorganisms Clarified. The extent of relatedness between microorganisms in several groups has been resolved through determination of DNA homology. Studies with additional species of Hansenula and Pichia showed 75% DNA relatedness between H. minuta and P. lindneri. This confirms our earlier work with other species of these two genera and demonstrates that their separation on the basis of ability to utilize nitrate as a sole source of nitrogen is invalid. By contrast, strains of the species Bacillus circulans were found through DNA studies to represent no fewer than nine distinct species thus demonstrating the inadequacy of present cultural and physiological tests for separating species. (See F, B.1.)

Fermentation of Cellulosic Intermediates Directly to Ethanol. The use of C. wickerhamii has potential in the production of ethanol from pretreated biomass residues. With current methodology, glucose can be generated from cellulose by either enzymatic saccharification or acid hydrolysis. Acid

hydrolysis is relatively rapid; however, if high glucose concentrations are generated in the presence of high acid concentrations, unfermentable reversion products, such as isomaltose, are formed. In addition, if the acid hydrolysis of bioresidues is not controlled properly, byproducts toxic to microorganisms are generated. However, if milder hydrolysis procedures were developed such that celloextrins were the major product, the formation of reversion products and toxic byproducts might be reduced. C. wickerhamii could then be utilized to produce ethanol from the soluble celloextrins. (See F, B.4.)

Fermented Feedlot Waste Can Be an Ingredient in Cattle Feed. In a 78-day feeding trial, fermented feedlot waste-corn proved acceptable to finishing cattle. The fermented diet, 11% waste on a dry basis, was supplemented with half the amount of oatlage and soybean meal used in the standard diet. Animals on the fermented diet ate 1.7 lb/day more (19.4 vs. 17.7) and gained 0.21 lb/day more (2.63 vs. 2.42) than animals on the control diet. Feed efficiencies were similar (7.38 fermented vs. 7.33 control). (See F, C.2.)

U.S. vs. Japanese Soybean Varieties in Making Tofu. The general impression that U.S. soybeans are not suitable for making Oriental soybean foods has been disproved. We recently studied tofu making from five U.S. and five Japanese soybean varieties grown in the same location and environmental conditions. There were no overall product differences associated with country or origin, although significant varietal variations was observed. Furthermore, soybean variety does not seem to play an important role in tofu processing; the processing conditions, on the other hand, greatly affect the yield and quality of tofu. For attractiveness and nutritional value of tofu, a variety with a light hilum and high-protein content is preferred. (See F, E.1.)

Occurrence of DON in Wheat from Areas Known to Have Scabby Wheat. The development of a more rapid reliable method for determining DON in wheat made possible a study of the trichothecene in hard red winter wheat collected in areas where scabby wheat had been identified. It was important to obtain the results as soon as possible in order to assess how serious the problem of scabby wheat could be in the export market. Hard red winter wheat in the better grades did not have levels of DON that would cause concern, considering the proposed FDA levels of concern. Correlations between the usual grading factors and scab damage and toxin levels were studied; the factors having the highest correlations were total damaged kernels, total defects, and scab-damaged kernels, including shrunken and broken kernels. The analyses were completed within a period of 2-½ weeks after the first samples were received. (See F, F.2.)

Cattle-Feed Trial with Naturally Aflatoxin-Contaminated Corn. In a cooperative project with the ARS National Animal Disease Center, Ames, IA, five steers were fed a noncontaminated diet; 10 steers were fed rations containing aflatoxin levels which increased from 350-450 ng/g during the study. Five of the 10 steers were taken off the contaminated rations at 15 weeks and fed

the noncontaminated diet the last 2.5 weeks. The remaining five steers were on the contaminated diet the entire 17.5-week period. All steers were killed and tissues and fluid samples were taken for analysis. All tissues and fluids taken from steers that remained on the aflatoxin-containing diet the entire period had B₁ and/or M₁ present (0-0.37 ng B₁/g, 0.11-4.82 ng M₁/g) with the most total aflatoxin in the kidney (5.91 ng/g) and liver (1.44 ng/g). In the animals switched to noncontaminated rations at 15 weeks, aflatoxins B₁ and M₁ had disappeared from all tissues and fluids except rumen contents (0.09 ng/g) at the time of sacrifice. Urine assays provided an indication of whether aflatoxin was present in the tissues. (See F, F.2.)

Successful Reanalysis of Feeds Used in Animal Feeding Trials of Ammoniated Aflatoxin-Containing Corn. Mixed feeds and corn used in feeding trials to obtain FDA approval for the process of detoxification of aflatoxin-contaminated corn by ammoniation were reanalyzed. Samples of chicken rations, swine rations, corn used in cattle-feeding studies, and rat chow rations prepared from tissues of animals used in feeding trials were analyzed by the NRRC method for mixed feeds and the CB method for corn. The NRRC mixed-feed method was successfully applied to all feeds including rat chow rations. The analytical results indicated that the assays done at the Russell Research Center during the time of the feeding trials were reliable. The accuracy and reliability of the original analyses are necessary to obtain FDA approval of the detoxification process for aflatoxin-containing corn by ammoniation. (See F, F.2.)

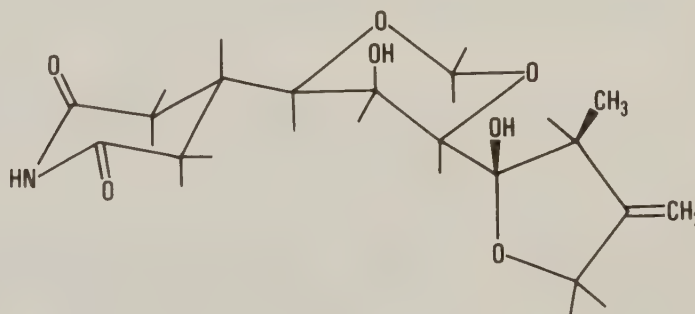
Aflatoxins in Conidia and Sclerotia of A. flavus and A. parasiticus. Substantial aflatoxin levels were recorded in fungal conidia and sclerotia that are components of the grain dust to which agricultural workers are exposed. Aflatoxin G₁ was detected in sclerotia of all A. flavus strains examined, but not in the conidia. In A. parasiticus, aflatoxin G₁ is present in both the sclerotia and conidia of all strains examined. Current evolutionary theory predicts an increase in the chemical defense systems of fungal sclerotia, propagules critical to fungal survival. (See F, F.2.)

Vomitoxin in Swine Feed. The trichothecene vomitoxin or 4-DON has been found widely distributed in corn, barley, wheat, and animal feeds. From January through July 1980, swine operations in the Midwest were confronted with lack of growth of feeder pigs and reproductive problems with sows. Additional signs noted were refusal to eat, vomiting, diarrhea, poor skin condition, and grinding of teeth. Of the 57 feed samples representative of the feed batches used at these swine co-ops during this 6-month period, 61% were found to contain vomitoxin in amounts ranging from 0.01 to 40 µg/g and many contained zearalenone. Of the 22 samples that did not contain detectable vomitoxin, 20% of these exhibited toxicity in bioassay systems. Microbiological examination of these 57 feed samples indicated a high number of *Fusaria* propagules in vomitoxin-positive samples. (See F, F.3.)

Sclerotium Germination in A. flavus and A. parasiticus. Sclerotium germination in A. flavus and A. parasiticus is sporogenic with conidial apparatus (yellow-green conidial heads) produced directly from exposed sclerotium surfaces. Germination occurs within 48-72 hr incubation (25-37°C) on moistened nonsterile soil. Because yellow-green conidia represent the infective inoculum when applied to corn silks, control of preharvest A. flavus infection may require agronomic practices designed to reduce numbers of sclerotia in field soils. (See F, F.4.)

Salt- and Heat-Tolerant Xanthanase. An enzyme complex that degrades xanthan gum (polysaccharide B-1459) was obtained from a Bacillus that is tolerant to salt (4% NaCl). In contrast to a similar complex formed by a Bacillus isolated from a low-salt environment, the salt-tolerant enzymes displayed stability to elevated temperature (45-48°C) in the presence of salt. This enzyme might serve as a viscosity breaker for hydraulic fracture proppants, based on xanthan gum, that are used for enhanced recovery of natural gas. The oil and gas recovery division of a gum producer is making a large batch of the enzyme for such use in a field test.

Characterization of Sesbanimide. From seeds of Sesbania drummondii, a new bioactive compound, sesbanimide, has been isolated and characterized. Sesbanimide has the following unusual structure:



Sesbanimide is highly active as an antitumor agent in experimental tumor systems. Sesbania drummondii and two other Sesbania species are noxious weeds on the Gulf Coast and southeastern USA, and have a long history of toxicity to livestock. It is probable that sesbanimide and related compounds are responsible for this toxicity. [See Horticultural and Special Crops Laboratory (HSC), B.2.]

A Photosynthesis Control Point Identified. Illumination conditions that photochemically reduce electron carriers in chloroplasts can result in the phosphorylation of chloroplast proteins and produce changes in the distribution of adsorbed energy between the two photosystems that operate in chloroplasts. Reasons for this phosphorylation and its effects are subjects of investigation in a number of laboratories worldwide. NRRC collaboration with D. J. Kyle (Michigan State University) discovered that phosphorylation produces a dramatic effect on the primary photochemistry of photosystem II,

which removes electrons from water to yield oxygen. In some way, photophosphorylation stabilizes the reduced form of a special plastoquinone molecule in the mechanism that shuttles electrons away from photosystem II. The accumulation of charge that accompanies this stabilization effectively inhibits photosystem II operation. Whether it also actively changes the distribution of absorbed quanta to preferentially excite photosystem I remains to be seen. (See HSC, C.2.)

Understanding Triglyceride Behavior. Natural fats and oils contain triglycerides which can exhibit different physical properties in the solid state and a variety of solid-solid-liquid transformations that have been poorly understood. These complex molecules can assume manifold configurations but it has proven possible, through computer assisted calculations, to develop spatial models which are leading to a detailed understanding of these phase transitions and other physical properties of the triglycerides. Subtle changes in molecular configuration are readily seen, through these calculations, to perturb molecular interactions substantially, so that properties depend not only on temperature but on the thermal history of the sample. The methods developed in this work are proving to be useful in resolving many long standing problems in triglycerides and the methods should be broadly applicable to a wide range of both physical and chemical problems. (See HSC, D.1.)

Tristearin Polymorphs Defined. Growth rates of plants and microorganisms and properties of commercial fats are highly dependent on the distribution of constituent lipid molecules among different physical states. Changing distributions produces profound effects; e.g., thermal death and fat bloom, by uncertain mechanisms. Detailed knowledge of the intrastate phases of lipid mixtures is lacking and even the description of polymorphic phases of pure lipids has been marked by uncertainty and controversy. A discovery of dependable methods for selective interconversion of tristearin into four distinctly different solid-state phases (α , β'_2 , β'_1 , β) and the characterization of these phases by Raman, infrared and x-ray now eliminates uncertainty with respect to multiple forms of tristearin. The ability of tristearin to adopt two stable β -prime forms, which was first observed here some years ago by differential scanning calorimetry, is confirmed. Furthermore, the results stand corroborated and accepted by laboratories at Lund, Sweden, and Thiars, France. Investigations of triglyceride polymorphism can thus focus on mechanisms controlling the interphase transitions. (See HSC, D.1.)

Discovery of a Soybean Germplasm Line Low in Linolenic Acid. Analysis of both the Northern and Southern soybean germplasm collections for fatty acid composition was completed this year. About ten soybean varieties were shown to have 5% or less of linolenic acid (18:3), which affects the oil stability leading to a variety of problems particularly troublesome in food use. Plantings of several of these lines at several locations showed one, PI 361088B, to be consistently low (4.4%) in 18:3. This variety was increased in Illinois and had a 18:3 content of 4.3%. Oil from five bushels obtained

will be tested, for its chemical properties. It has not yet been determined whether this reduction of 18:3 from 7-10% down to 4.3% is an adequate improvement, but this line, PI 361088B, is a significant stepping stone on the route to improved soybean oil quality. (See HSC, D.2.)

Nitrogen Isotope Ratio Analysis Rate Increased. Significant modification in equipment and computer software allows analysis of 200 samples of ammonium chloride for $^{15}\text{N}/^{14}\text{N}$ ratio per day. This is almost a threefold increase over the previous sample rate. This will allow NRRC to take some of the sample load previously analyzed outside of ARS and contribute to the accomplishment of the mission of the N_2 X tillage national program. (See HSC, E.1.)

Detection of Fusarium Mycotoxins by MS/MS. A newly acquired "tandem spectrometer" (MS/MS) promises to expedite many analyses including mycotoxins. In effect, the first MS component achieves isolation and the second one, analysis. The Fusarium mycotoxins DON and zearalenone can be detected rapidly in a crude extract of grain by quadrupole MS/MS analysis. The two toxins (0.1-ppm each) were detected in a single analysis. These results indicate the possibility of simultaneous analysis of a number of mycotoxins with substantial additional time savings resulting from avoiding cleanup and derivatization steps. (See HSC, F.2.)

Secondary Lipid Oxidation Products and Their Decomposition Pathways. The oxidation of linolenate in soybean oil is a key process leading to the flavor deterioration and loss of nutritional value of this major world food fat. We have now established the formation of new bis-cyclic peroxides related to prostaglandin endoperoxides in methyl linolenate oxidized with singlet oxygen. Four isomeric 5-membered bis-cyclic peroxides were separated by high-performance liquid chromatography and were shown by nuclear magnetic resonance and mass spectrometry to have a unique epidioxide structure. The structural evidence obtained on these secondary oxidation products has now been used to clarify their mode of decomposition. A better mechanistic understanding of the flavor precursors and their decomposition pathways will lead to improved methods of controlling rancidity in lipid-containing foods. [See Oilseed Crops Laboratory (OC), A.2.]

Mass Spectrometry (MS) of Phospholipids. The determination of phospholipid molecular species by conventional techniques is a time-consuming multi-step process which requires comparatively large samples and carefully controlled conditions. Intact phospholipids have proved refractory to MS analysis because of their low volatility and tendency to decompose on heating or in the electron beam. The technique of chemical ionization MS has now been shown to provide molecular species identification and reduce analysis time from hours to minutes. (See OC, A.3.)

Soybean Calmodulin. Calmodulin is a protein that binds calcium and is an important regulator of cellular processes. Electrophoretically homogeneous calmodulin has now been isolated from soybeans and purified 275-fold by

procedures including ion-exchange, affinity chromatographics, and high performance liquid chromatography. Calmodulins previously isolated from other sources consistently contain 9 or 10 methionine residues per molecule whereas soybean calmodulin contains only 1 or 2 methionines/mole. A comparative study of soybean and bovine calmodulin indicates that soybean calmodulin has a lower specific activity with respect to its ability to stimulate calmodulin-deficient phosphodiesterase and differs in its effect on embryonic chick brain cholinergic receptor binding as compared to bovine calmodiolin. (See OC, A.4.)

HPLC Analysis Shows Fate of Nightshade Glycoalkaloids in Soy Processing. In 1981, soybeans grown in the upper Midwest were found to contain Eastern black nightshade (Solanum ptycanthum) berries. Five glycoalkaloids in nightshade have been identified and high performance liquid chromatography (HPLC) has shown the following contents (mg/g): β -solamargine 2.4, α -solamargine 3.3, α -solasonine 4.9, α -chachonine 05, α -solamine 1.0. The glycoalkaloid content of soybean protein products prepared from soybean-nightshade blends show a generally uniform distribution of the alkaloids in all fractions except for a protein concentrate made by the alcohol extraction process which contained only small amounts of alkaloids. Thus the glycoalkaloids are not removed when contaminated soybeans are processed into defatted flours and isolates by conventional processes. (See OC, A.4.)

Solubility of Seed Oils in Supercritical Carbon Dioxide. Contrary to theory based on present understanding of solubility parameters and to theory based on observations of previous researchers, at temperatures above 60°C the solubility of seed oils in SC-CO₂ increases dramatically to well over 20% by weight with increasing pressures above 8,000 psi. This rapid response of solubility to temperature and/or pressure allows much faster extraction rates and affords more economical methods for recovery of the oil. A major portion of the extracted oil can be removed from the process stream by slightly decreasing the temperature at constant pressure or the pressure at constant temperature. (See OC, A.5.)

Vegetable Oil Microemulsion Fuels. Microemulsions consisting of aqueous alcohol dispersed in vegetable oils are being considered as alternative fuels that will permit crop production during a petroleum shortage. Such microemulsions have viscosities much closer to engine design specifications and could improve combustion properties compared to the original vegetable oils. For the first time, it has now been shown that 1-butanol can serve as a microemulsifying agent. Conductometric, viscometric, and ultracentrifugal data confirmed the microemulsion structure. The microemulsion fuel has been tested and has given encouraging results in cooperative work conducted by North Dakota State University and the University of Illinois. (See OC, B.1.)

Ultrasonic Hydrogenation of Vegetable Oils. NRRC scientists recently discovered that ultrasonification markedly increases the rates of hydrogenation of soybean oil with both nickel and copper catalysts. For example, 87% of the linolenate in soybean oil was hydrogenated in 8.6 seconds at 181°C,

115 psi pressure when copper catalyst was used with ultrasonic energy. Without ultrasonic energy, 480 seconds were required to hydrogenate only 68% of the linolenate at 200°C. Similar results were obtained with nickel catalyst. (See OC, C.1.)

Preparation of Geometrical Isomers of Methyl Linolenate. Linolenic acid and its geometrical isomers are examples of dietary n-3 fatty acids which are recognized to have important nutritional effects on the biosynthesis of prostaglandins and leukotrienes. The development of a new general synthetic scheme and a rapid purification procedure has made possible for the first time the preparation of all eight linolenic acid geometrical isomers and has wide applicability to the preparation of other fatty acids and insect pheromones. The availability of these n-3 isomers is important in order to allow investigations of the nutritional and metabolic characteristics of these isomers which are formed during hydrogenation and deodorization of soybean oil. (See OC, D.1.)

Reverse Osmosis and Ultrafiltration Reduces Costs of Alcohol. Savings of considerable energy and 5 to 6 cents per gallon of alcohol can be achieved if distillers' solubles are concentrated by membrane technologies instead of conventional drying during ethanol production. When mashed grain is fermented to alcohol, a residue (stillage) that contains only 5 to 10% solids remains after the alcohol is distilled off. This stillage is screened or centrifuged to remove most of the suspended insoluble solids. The remaining dilute soluble fraction is usually concentrated by evaporation and considerable amounts of energy and cost are expended. Reverse osmosis separates water from a solution by means of a membrane that is more permeable to water than to dissolved matter. The water is pumped at high pressure through the membrane to overcome the osmotic pressure that opposes such migration. Since no evaporation of water is involved in reverse osmosis, energy consumption is much lower than in concentration by heating. Use of ultrafiltration, a process that removes larger molecules such as proteins by means of semipermeable membranes, prior to reverse osmosis extends the concentration range for stillage solids suitable. Use of the combined techniques facilitated concentration of recycling distillers solubles resulting in further energy savings for reverse osmosis by preventing clogging of the reverse osmosis membranes. (See Northern Agricultural Energy Center, A.4.)

BIOMATERIALS CONVERSION LABORATORY

W. M. Doane, Chief

Research Leaders: R. A. Anderson, R. J. Bothast, M. E. Carr,
G. E. Hamerstrand, and F. H. Otey

A. PHYSIOLOGICAL AND BIOCHEMICAL TECHNOLOGY TO IMPROVE CROP PRODUCTION

1. Ion Transfer in Photosynthesis (Cooperative Agreement - University of Notre Dame)
 - a. Specific Objective: Investigate the relationship between trans-membrane H⁺ gradients and ATP formation in higher plant chloroplasts and in reconstituted systems.

Progress: The flux of protons in artificial proteoliposomes comprised of soybean phospholipids and the light-driven H⁺-pump bacteriorhodopsin was investigated by entrapping one or more water soluble, pH sensitive fluorescent dyes within the vesicles. Internal acidifications resulting from illuminations as short as 10 ns (laser) could be resolved kinetically ($\tau_{1/2} \sim 10$ ms). When two probes of different pKa were entrapped within the same vesicle preparation, it was also possible to determine the extent of reconstitutive heterogeneity. H⁺ efflux from the proteoliposomes was always faster than other ion fluxes, and was accelerated dramatically when the chloroplast ATPase complex was included in the membranes.

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B. TECHNOLOGIES FOR FOOD AND FEED USES OF FIELD CROPS

1. Fundamental Studies on Separation of Starch, Protein, and Lipid of Corn (R. A. Anderson)

- a. Specific Objective: Investigate relationship of basic corn milling procedures to kernel characteristics, breakage susceptibility, and varietal differences.

Progress: Dry milling studies were conducted on six corn hybrid varieties from the 1981 crop representing combinations of 10 different genotypes and the resulting products were chemically and physically characterized. Upon harvest, each sample was dried at 77 and 140°F. Kernel breakage (Stein test) and stress crack count increased for every variety dried at the higher temperature as compared to the 77°F temperature. Breakage was highest (7-9%) in the variety with the lowest nitrogen content. The two varieties containing the greatest amount of crude fat (4.5 and 5.0%) had the lowest linoleic acid contents (55 and 60%). For all corn varieties, fat content varied inversely with kernel size. Kernel equilibrium moisture content decreased in corn dried at 140°F. Degermer thruputs in the milling of corns dried at 140°F increased over that of corns dried at 77°, but first break grit yields were less from the corns dried at 140°F compared to that of corns dried at 77°F. As with studies on 1980 crop year corn, 1981 varieties showed little or no correlation between kernel hardness and breakage and/or milling. The major effects were apparently caused by the difference in drying temperatures.

Experimental corn dry milling studies were completed to establish a shortened mill flow, which would reduce a number of operations in the milling process and provide raw materials for possible alcohol and/or starch production. This work indicated that low-fat grits could be produced without going through the elaborate tempering procedures and roller milling processes now being used. Reducing corn temper moisture and temper time prior to degerming is feasible for production of low-fat grits, but with low-moisture, short-time temper conditions the attached hull content is markedly increased. For more refined uses of corn grits this would be objectionable, but for the applications suggested above some attached hulls should offer few or no problems.

- b. Specific Objective: To determine whether SC-CO₂ extraction of corn endosperm fractions will remove lipoidal proteinaceous material and

thereby improve the dry separation of the remaining starch and protein.

Progress: Lipoidal constituents identified as zeaxanthin, lutein, and β -carotene can be partially extracted from grits, meal, and flour with supercritical CO₂ (SC-CO₂) but particle size and lipid solubility limits yield. However, under these extraction parameters (10,000 psig and 65°C) physical changes in the matrix protein as observed microscopically (SEM) appears to effect fragmentation. Endosperm agglomerates can be ground into a fine powder (>100 microns) containing some free starch granules. Preliminary studies on use of ethanol as an entrainer during extraction increased lipid solubility significantly. This exploratory work with SC-CO₂ extraction shows potential for producing a high β -carotene oil for poultry feed and crude starch fractions for gasohol, high fructose syrup and other industrial use.

- c. Specific Objective: To explore the effect of higher CO₂ pressure (to 30,000 psi) on the oil extraction of wet milled germ and on the flavor and stability of the residual meal.

Progress: CO₂ permeability through cell walls and oil sacs of corn germ is greatly reduced if the germ is SO₂ processed. In contrast, oil recovered from dry milled germ is excellent (99%). Even when wet milled germ is air dried or lyophilized to low moistures, oil yields are less than 90%. However, oil yields might be improved by increasing extraction pressures. Equipment to study higher pressures has recently been installed.

- d. Specific Objective: To extract lipids from cereal starches and investigate the effect of this extraction on the gelatinization characteristics of the residual starch.

Progress: Differential scanning calorimetry of SC-CO₂ extracted corn starch demonstrates partial extraction of lipids but the lysolecithin that affects gelatinization is not extracted. No change in gelatinization characteristics or rheological properties could be shown with SC-CO₂ corn starch when compared with native corn starch.

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2. Nutritional Quality, Safety, and Flavor Aspects of Soybean Protein Products (R. A. Anderson)

See Oilseed Crops Laboratory, A.3.

C. BIOMATERIALS SCIENCE

1. Plant Component Separation and Physical Characterization (T. P. Abbott)

- a. Specific Objective: Determine the nature of biodegradation products of kenaf lignin with Cyathus species.

Progress: Kenaf plants with ^{14}C labelled lignin were degraded with 12 different species of Cyathus and the rate of CO_2 generation, rate of water soluble species generation, and the molecular weight distribution of soluble species were determined and the data are being summarized.

In related work, the lignin-carbohydrate complex generated by Cyathus stercoreus on wheat straw was found to contain 48% polysaccharide consisting of arabinose (23%), xylose (12%), galactose (6%), mannose (5%), and glucose (1%).

- b. Specific Objective: Continue studies to determine the chemical mechanism of wheat straw and kenaf lignin degradation by Phanerochaete chrysosporium.

Progress: Studies with model oxidants indicated that H_2O_2 can be an effective delignifying agent under certain conditions, which were characterized. Materials such as oak, kenaf, wheat straw, and corn stalks were delignified by H_2O_2 at $\text{pH} > 10$ and room temperature in only a few hours. The insoluble residue, which was rich in cellulose, was easily converted to glucose, which could be further fermented to ethanol with high overall efficiency. Attempts to identify significant levels of H_2O_2 in liquid cultures of P. chrysosporium have been unsuccessful, however. New model studies on the possible role of singlet oxygen in biodelignification have been initiated.

- c. Specific Objective: Initiate new studies on the electronic, thermal, and physico-chemical properties of insoluble polymers and other plant materials using photoacoustic spectroscopy (PAS).

Progress: A complete, dual channel UV-visible PAS system was constructed, tested, and has been utilized to characterize the light-absorption properties of in situ lignin in wheat straw and a variety of woods. It was discovered that high molecular weight lignin in situ is often significantly modified, apparently as the result of photochemical oxidation, into more highly conjugated forms which absorb light in the visible region, giving these materials their characteristic yellowish colors. Model reactions using thermomechanical pulps and newsprint indicated that near UV light absorption by lignin, and the subsequent generation of singlet

oxygen, were primarily responsible for these chemical modifications. In order to extend the capabilities of the PAS system, gratings for near IR studies were purchased, as well as a Fourier Transform IR spectrometer equipped with a PAS cell.

- d. Specific Objective: Investigate the effect of physical, chemical, and/or biological treatments of cellulosic materials in high-shear reaction systems on their composition, properties, and reactivity for new and improved uses of the modified substrates.

Progress: Wheat straw (WS) was subjected to a variety of chemical, physical, and mechanical treatments in a continuous mixer (Model 50 Ko-Kneader) to investigate rapid, low-energy, and continuous chemimechanical methods of disrupting the lignin-hemicellulose-cellulose complex. Several alkaline systems were quite effective for removing lignin and pentosans from WS as water-soluble components without cellulose degradation in reaction periods of only 3 to 6 minutes (98°C). For example, 58% of the WS lignin and 32% of the WS pentosans were removed with the use of 15.5% sodium hydroxide (dry straw basis), a 6-minute period in the reaction chamber, and an agitator speed of 24 rpm. With the same amount of hydroxide 63-66% lignin and 41-43% pentosans were removed in 3-4 minutes with agitator speeds of 35-93 rpm (various production rates). Incorporation of 0.3% anthrahydroquinone with 15.5% sodium hydroxide removed 70% lignin and 39% pentosans. The addition of 5.0% sodium sulfide and 12.7% sodium hydroxide (total effective alkali equivalent to 15.0% sodium hydroxide) removed 43% pentosans (32% for 15.5% sodium hydroxide alone). Hydrogen peroxide (2% dry straw basis) was not effective in a 6-minute reaction period for removing lignin and pentosans under alkaline or neutral conditions with or without ferrous ammonium sulfate (1.4% Fe, dry straw basis). Analyses of WS treated with hexamethylenetetramine and 1,6-hexanediamine have not been completed. Experiments to support the significance of chemical and mechanical interaction in these reactions and to assess the effect of the treatments on accessibility of the residual cellulose to enzymatic hydrolysis are in progress.

- e. Specific Objective: Investigate new concepts for the separation of cellulose, hemicellulose, and lignin components of biomass.

Progress: Wheat straw was irradiated with cobalt-60 in the presence of acrylic acid; however, neutralization of the resulting graft copolymer with alkali followed by high-speed stirring did not disintegrate the straw particles and did not produce a significant quantity of water extractables. A graft copolymer of wheat straw was similarly prepared from AMPS (a sulfonic acid-containing monomer). Refluxing this graft copolymer in water for 22 hours resulted in little hydrolysis of the polysaccharide components. Milled wheat straw was refluxed with dilute trifluoroacetic acid (TFA) to

selectively hydrolyze the hemicellulose component to xylose and xylodextrins. Xylodextrins could be isolated after a 1 hour reflux with 0.05N TFA. A 2 hour reflux with 0.1N TFA resulted in a 28.7% weight loss, and xylose was present in the soluble fraction.

- f. Specific Objective: Initiate new studies on the handling, storage, and preservation of biomass such as sweet sorghum.

Progress: Two sweet sorghum varieties, Wray and Keller, were successfully grown at several locations in Central Illinois. After harvesting, stalks (30% sugar, dry basis) and bagasse (10% sugar, d.b.) were stored using two methods. Bagasse was stored outside in sealed containers (anaerobic conditions) with no treatment, with propionic acid (5% d.b. wt/wt), and with a CO₂ atmosphere (100%). Stalks were stored outside in sealed containers with no treatment, with propionic acid (6% d.b. wt/wt), with NH₃ (aqueous, 4% d.b. wt/wt), with SO₂ (6% d.b. wt/wt), and with a CO₂ atmosphere (100%). After 200 days of storage, only the stalks from the SO₂ and propionic acid treatments had significant levels of sugar remaining, 10% and 18% sugar, d.b., respectively. All others were in the range of 1-2% sugar (d.b.).

Stalks were also stored outside (aerobic conditions with minimal protection), with no treatment (horizontal position), with no treatment (vertical), after drying to 50% moisture (horizontal), and with propionic acid treatment (horizontal). No detectable sugars were found after 200 days of storage. It should be noted that both these aerobic and anaerobic tests were severe storage tests with long storage periods and widely varying weather conditions (e.g., temperatures from -10 F to 80 F).

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2. Basic Studies on Modification of Natural Polymers as Replacements For Petroleum-Derived Polymers (F. H. Otey)

- a. Specific Objective: Continue the study to develop methods and mechanism to modify biopolymers to provide an expanded knowledge base for replacing petroleum-derived polymers with natural polymers.

Progress: Since earlier studies demonstrated that blending of synthetic polymers with natural polymers greatly improved the flexibility of natural polymers, this study was designed to evaluate the effects of both blending and chemical combinations of synthetic materials. Techniques were developed and applied to the grafting of acrylonitrile, methyl acrylate, and methyl methacrylate onto starch at levels of 5 to 20% graft. In addition, natural polymers were pretreated with various levels of alkali and plasticized with polyols as a further approach to controlling the stability of natural polymer systems.

- b. Specific Objective: Continue a basic program to determine effects of new and novel physical and chemical modifications on the physical properties of biopolymers in thermoplastic applications.

Progress: To replace petroleum-derived polymers with natural polymers, it appears essential to overcome the moisture sensitivity and tendency of natural polymers to associate with themselves forming a high crosslinked density resulting in brittle structures. To determine the effects of various modifications on these deficiencies, the evaluation program was expanded to include aging and water soaking effects on stress-strain characteristics of composite systems containing the natural polymer. Thirty-two composites containing 20 to 50% modified and unmodified starches and 80 to 50% ethylene-acrylic acid (EAA) were extrusion blown into films and tested on an Instron (before and after 1 hour water soaking) one and six months after preparation. Films made with unmodified starch and EAA showed no significant loss in tensile strength or percent elongation after one and six months aging with and without water soaking. Maximum shrinkage due to 1 hour water soaking was only 2%. These data support earlier observations that

EAA prevents gelatinized starch from reassociating into a brittle polymer. The addition of 5% (based on starch) of polyol plasticizer significantly improved percent elongation of these systems but much of this added polyol was removed during water soaking. However, such additives may improve rate of biodegradation. The most promising modification was gained by pre-treating the starch with alkali. This treatment appeared to improve compatibility between EAA and starch, and increased tensile strength and percent elongation by 30%. Even though the alkali is removed during water soaking, the resulting films retain certain characteristics that deserve further evaluation. Grafting various monomers onto starch did not appear to offer sufficient property improvements to justify further study on this approach.

- c. Specific Objective: Establish cooperation with academia, industry, and government scientists.

Progress: Ten samples of plastic film were made by extrusion blowing that contained various levels of starch, super slurper, calcium chloride, and antibacterial agents. These samples were supplied to a cooperator where scientists are evaluating the effects of biopolymers on the migration of the agents for possible medical applications. Results of these tests have not yet been reviewed. Thirteen samples of biopolymer film were prepared and sent to the University of Illinois for evaluation as potential mulch. Preliminary results of these tests suggest that two of the formulations are promising for mulch applications.

- d. Specific Objective: Initiate new studies to determine which of a wide variety of modifications to biopolymers impart significant soil-aggregate stabilizing activities.

Progress: Three types of polyanionic starch were prepared: carboxymethyl starch, starch-g-polyacrylic acid, and hydrolyzed starch-g-polyacrylonitrile. Each class of derivative was prepared at five levels of anion content and soil particles were treated with several concentrations of dispersions of each polymer and then wet sieved to measure soil particle stabilization. On a weight basis, the starches did not stabilize as well as their wholly synthetic counterparts but on a meq COOH/100 g soil basis, the grafted starches performed as well or better.

- e. Specific Objective: Continue a systematic approach to find an optimum method for plasticizing starch film with the ultimate aim of establishing a correlation between the chemical structure of polyols and other classes of organic compounds and their effectiveness as starch film plasticizers.

Progress: The synthesis of the five triol polyols needed for the systematic correlation of polyol structure to starch film

plasticization effectiveness has been completed. A manuscript note on the synthesis is being prepared. A survey on the literature on the infrared absorption spectra of polyols (aliphatic) has been completed and a manuscript based on the infrared data of the polyols being used in this program is also being drafted.

- f. Specific Objective: Study the variables in extrusion processing of starch graft copolymers having glassy, high Tg grafted branches.

Progress: When a semi-solid mixture of starch, styrene, water, and potassium persulfate in a weight ratio of 100:50:25:1 was heated (nine repetitive experiments), an exothermic polymerization took place to give a reaction product that contained 26-28% polystyrene, the major portion of which was grafted to starch. Conversions of styrene to polymer ranged from 68-79%, and the grafted polystyrene amounted to 82-88% of the total polystyrene formed, the remaining 12-18% being benzene-soluble homopolymer. Benzene-extracted products contained 24-26% grafted polystyrene with mean M_w and M_n values of 515,000 and 190,000, respectively. Removal of a portion of the carbohydrate by enzymatic hydrolysis produced a graft copolymer containing 40% polystyrene; however, this polystyrene content was not high enough to permit processing by extrusion. Hydrolysis with 0.01 N hydrochloric acid produced a graft copolymer containing 58% polystyrene. This polymer was easily extruded to yield a strong continuous plastic with an ultimate tensile strength of 4500 lb/in². Plasticizing the polystyrene component with 15 p.h.r. dibutyl phthalate reduced the tensile strength to about half this value, while plasticizing the starch component with glycerol reduced the tensile strength by about a factor of 10.

- g. Specific Objective: Study the use of biomass components as substrates or templates to facilitate the preparation of difficult to synthesize block copolymers.

Progress: Starch-g-polyacrylonitrile (PAN) copolymers were prepared by ceric ammonium nitrate initiation, and the major portion of the starch in these graft copolymers was then removed by acid hydrolysis to yield PAN with oligosaccharide end groups. Although these PAN-oligosaccharide samples reacted with methyl methacrylate in the presence of ceric ammonium nitrate, the resulting products were largely graft copolymers rather than the expected PAN-poly(methyl methacrylate) (PMMA) block copolymers. The following evidence is presented for a PAN-g-PMMA structure: (a) PAN without oligosaccharide end groups also produced a copolymer with methyl methacrylate under our reaction conditions. (b) Starch-g-PAN (51% or 37% add-on) was a less reactive substrate toward ceric-initiated polymerization than PAN with oligosaccharide end groups. (c) Low add-on (18%) starch-g-PAN reacted with methyl methacrylate to give a final graft copolymer in which a large percentage of PMMA was grafted to the PAN component rather than to starch.

- h. Specific Objective: Study the reaction of guaiacylglycerol- β -guaiacyl ether with acetylene.

Progress: The model compound was synthesized and soluble reaction products, polymer, and two dimers have been isolated and partially characterized.

- i. Specific Objective: Investigate the feasibility of both separate and simultaneous saccharification and fermentation of liquefied starch with a continuous-flow system of encapsulated microorganisms.

Progress: Three methods were investigated for converting starch to ethanol using continuous flow of liquefied starch (LS) through columns of immobilized (entrapped) microorganisms. The continuous-flow system apparatus and methods of Ca-alginate cell immobilization were developed previously and reported in NRRC studies. Native corn starch was batch liquefied and converted to ethanol at 35°C by the following three methods. (1) LS was pumped continuously through a column of immobilized glucoamylase and then a column of immobilized commercial yeast. (2) LS was pumped through a column of immobilized mixtures of glucoamylase and yeast (simultaneous saccharification/fermentation, SSF). (3) LS was batch saccharified with glucoamylase to approximately a theoretical yield of glucose and then pumped through a column of immobilized yeast. The primary materials for all experiments were Taka-Therm (alpha-amylase), Diazyme (glucoamylase), Fleishman's yeast, and sodium alginate converted to the calcium matrix. Variables examined included pH, temperature, flow rate, and amounts of glucoamylase, yeast, and alginate. Samples were collected daily for two weeks in each experiment to determine amounts of glucose and ethanol. Yields of ethanol and longevity of the yeast cells were greatest for the batch saccharification/continuous fermentation and were least for the SSF. Similar experiments with cultures of Saccharomyces cerevisiae NRRL-Y2034 are in progress. A manuscript is forthcoming.

- j. Specific Objective: Correlate the physical properties of granular carbohydrate-g-poly(methyl acrylate) and related copolymers with the distribution of copolymer in the granules.

Progress: Normal range of observed tensile strength, ultimate elongation, extruder die pressure, extrusion rate, extruder die pressure, and extruder RPM values were determined for representative corn starch-g-PMA (polymethyl acrylate) samples. Samples examined by electron microscopy show that individual starch graft copolymer particles retain their identity throughout the extrusion but are deformed to the point where the starch granule portion as well as the PMA in the graft copolymer must flow. This is suggestive of deep penetration of the granules by the grafted PMA. PMA containing 1% of the functional tetramethylene glycol dimethacrylate as a cross-linking agent was grafted to granular starch to produce

infusible PMA which retains its identity and shape on removal of the starch so that scanning electron microscopy of split particles can reveal the depth of penetration of the PMA grafts into the granules.

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SWANSON, C. L., G. F. FANTA, AND E. B. BAGLEY. Particle Flow and Sintering Processes in Extrusion. Industrial Materials Research Institute Symposium Proceedings-Polym. Eng. Sci. In press.

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CARR, M. E., L. T. BLACK, AND M. O. BAGBY. Starch Liquefaction and Saccharification by a Continuous Process. Presented at the 16th Great Lakes Regional ACS Meeting, Normal, Illinois, June 7-9, 1982.

3. Basic Studies on Biopolymers for Improving Safety of Pesticides (F. H. Otey)

- a. Specific Objective: Investigate new methods for encapsulating pesticides that involve interactions of natural polymer derivatives with metallic salts and determine effects of these methods on the release rates of pesticides.

Progress: Several modifications of the starch-borate technique were developed. These include: use of a pregelatinized starch or flour with ammonia and boric acid to give a more moderate operating pH range, use of a detergent to decrease the amount of water necessary for the process, and pregelatinized corn flour to seal the capsules and improve pesticide retention. Evaporative tests performed with encapsulated volatile pesticides showed release characteristics comparable to release of similar pesticides in the xanthide, calcium adduct, and earlier borate methods. Both water soluble and water insoluble pesticides have been formulated by the starch-borate techniques.

- b. Specific Objective: Investigate the incorporation of modified natural polymers into spray applied tank mix systems as a potential approach to controlling pesticide release and reducing losses due to evaporation and decomposition.

Progress: Research was initiated to formulate new tank mix systems in order to control the release of thiocarbamate herbicides. Preliminary results indicate that when aqueous dispersions of gelatinized corn starch and pesticide are sprayed onto surface and allowed to dry, the pesticide is trapped within the starch matrix for controlled release. Further research is needed to optimize the conditions for such use.

- c. Specific Objective: Continue cooperation with academia, industry, and government scientists by formulating and providing suitable encapsulated samples for greenhouse and field evaluation.

Progress: Over 50 samples of encapsulated pesticides were provided to 15 collaborators from academia, industry, and government. Large scale field trials conducted at Purdue University agronomy farm demonstrated the efficacy of starch encapsulated trifluralin in late fall application for weed control the following spring. The trials also demonstrated that encapsulation of volatile herbicides such as EPTC allows incorporation to be delayed several days without loss of effectiveness. The newer developed borate procedures allowed us to supply collaborators with samples containing mixtures of water soluble and water insoluble compounds.

- d. Specific Objective: Continue studies to evaluate reaction variables to maximize encapsulation of pesticides into biopolymer matrixes.

Progress: When aqueous dispersions of pesticides and nonionic surfactants were sonified prior to encapsulation into a starch matrix, the pesticide was more uniformly distributed within the matrix and it released more slowly during accelerated aging. Also, the method of encapsulation affected pesticide release: starch xanthide > calcium-starch adduct > borate starch adduct.

- e. Specific Objective: Conduct toxicological studies on encapsulated pesticide formulations that show greatest promise.

Progress: Samples of starch-encapsulated ³⁵S-parathion were provided for Dr. Ronald T. Riley, Research Pharmacologist (ARS, Athens, Georgia) who completed the toxicological studies. Results indicated that the encapsulated parathion provided significant decreases in volatility, solubility, and percutaneous permeability of parathion as compared with commercial clay formulated samples containing similar levels of parathion.

- f. Specific Objective: Initiate studies to formulate mathematical expressions for the absolute rate of dissolution and/or diffusion of particular natural-polymer encapsulated pesticide compositions to provide a basis for understanding the relationship between composition variables and release rate of the pesticide.

Progress: Conventional theoretical principles of diffusion-controlled release in pharmacology have been shown to be applicable to starch xanthate-encapsulated trifluralin under laboratory conditions. Mathematical models which describe the kinetics of particular controlled-release pharmaceuticals may therefore provide a theoretical foundation for understanding the mechanism of release of pesticides from biopolymeric systems. Since mathematical optimization plays an important role in modeling, computer

optimization methods are being devised that should contribute to the success of formulating and testing the models. The computer algorithms have already been used to solve the problem of blending various compositions of encapsulated pesticide to achieve desired release rate patterns.

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WING, R. E. AND F. H. OTEY. Determination of Reaction Variables for the Starch Xanthide Encapsulation of Pesticides. J. Polym. Sci., Polym. Chem. Ed. In press.

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SHASHA, B. S. Encapsulation of Pesticides Within Starch Matrices. Lecture at the Conference of General Cooperators from the North Central Agricultural Experiment Stations, NRRC ARS/NCR, USDA, Peoria, Illinois, October 18-19, 1982.

4. New and Improved Technologies for the Fermentative Production of Chemicals from Biomass (R. J. Bothast)

- a. Specific Objective: Select bacteria that have potential for fermentative production of useful chemicals.

Progress: Bacterial strains (100) were examined on each of five substrates: Trypticase soy broth (TSB) without dextrose; TSB + 5% glucose; TSB + 5% xylose; TSB + 5% glycerol; and TSB + equal measures of glucose, xylose, and glycerol to yield a 5% carbon source. The fermentations were carried out in 100 ml volumes of substrate in 300 ml erlenmeyer flasks. They were shaken at 150 r.p.m. at 28°C for 6 days. Sampling was done at 0 time, 3 days, and 6 days. Samples were taken for pH, optical density, and HPLC analysis of chemicals produced. Analysis has shown that some bacterial strains produce useful chemicals. These promising strains have been selected for a more detailed examination of the chemical products produced.

- b. Specific Objective: Improve the production of β -hydroxypropionaldehyde from glycerol.

Progress: Conversion of glycerol to β HPA was attempted via action of glycerol dehydrase isolated from *Lactobacillus* sp. NRRL B-1720. This method, however, was unsatisfactory because enzyme activity was lost within 60 to 90 min of reaction initiation. Fermentation of glycerol by whole cells of *Klebsiella pneumoniae* NRRL B-199 in the presence of optimal semicarbazide hydrochloride proved more effective. Using this technique, 30 g/L glycerol solutions yielded 13.1 g/L β HPA solutions. Thus a conversion efficiency equal to 55% of the theoretical maximum was realized.

Publications:

SLININGER, P. J., R. J. BOTHAST, AND K. L. SMILEY. Production of β -Hydroxypropionaldehyde from Glycerol. Appl. Environ. Microbiol. (Submitted).

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5. Recovery of Rubber from Guayule and Related Whole Plant Materials
(G. E. Hamerstrand)

- a. Specific Objective: Finalize studies on analytical methodology for determining the rubber content of guayule shrub.

Progress: The accuracy and reproducibility of the gravimetric methodology for determining the quantity of resin and rubber in guayule have been evaluated in a collaborative study involving seven other laboratories. These included ARS Field Stations as well as universities in the United States, and two laboratories in Australia. Based on a preliminary evaluation of these data, it appears that the gravimetric method developed at NRRC in cooperation with the U.S. Water Laboratory in Phoenix, Arizona is the best method in terms of accuracy, reproducibility, and equipment expense for use in agronomic and developmental studies at the Field Station level.

- b. Specific Objective: Complete basic engineering studies, design, and construct a prototype solvent extraction process for removing both the resin and rubber from whole plant materials.

Progress: Each segment of the proposed pilot, solvent extraction process was examined, appropriate equipment and conditions selected, and the individual components integrated into a process that was evaluated for its applicability for extracting rubber from guayule and milkweed. Preliminary trials have demonstrated the technical feasibility of adapting and using counter current solvent extraction equipment, designed primarily for oilseed extractions, for processing guayule and related whole plant materials. At a feed rate of 25 pounds per hour and a 2:1 solvent:shrub ratio approximately 85% of the rubber contained in guayule could be efficiently removed. The process was also adaptable to other materials (those not requiring flaking) and was used to remove the resin and rubber from

approximately 200 pounds of milkweed in a cooperative study with another research organization.

Publication:

BLACK, L. T., G. E. HAMERSTRAND, F. S. NAKAYAMA, AND B. A. RIANIK. Gravimetric Analyses for Determining the Resin and Rubber Content of Guayule. Rubber Chem. Tech. In press.

6. Hydrolysis of Hemicelluloses by Enzymes from Anaerobic Bacteria (Cooperative Agreement - Virginia Polytechnic Institute and State University)

- a. Specific Objective: Develop new biochemical processes using enzymes from anaerobic bacteria to hydrolyze plant hemicelluloses to fermentable sugars for the production of chemical feedstocks.

Progress: The presence of enzymes capable of degrading crude hemicellulose, hemicelluloses A & B, and commercial xylan has been confirmed in three strains of Bacteroides ovatus grown on a medium containing wheat hemicellulose. The activity was mainly intracellular with about 17 percent enzymatic degradation of wheat straw hemicellulose fractions in 18 hours.

Publication:

REDDY, N. R., J. K. PALMER, M. D. PIERSON, AND R. J. BOTHAST. Wheat Straw Hemicelluloses: Isolation, Composition, and Fermentation by Human Colon Bacteroids. J. Agric. Food Chem. (Submitted).

7. Hydrocarbon-Producing Plants as Potential Multi-Use Crops (M. E. Carr)

See Northern Agricultural Energy Center, A.1.

8. Microbiological and Enzymatic Procedures for Conversion of Agricultural Residues and Biomass (R. J. Bothast)

See Northern Agricultural Energy Center, A.2.

9. Innovative Fermentation Technology for Alcohol Production (R. J. Bothast)

See Northern Agricultural Energy Center, A.3.

D. TECHNOLOGIES AND PRODUCTS TO INCREASE
EXPORTS OF AGRICULTURAL PRODUCTS

1. Principles Underlying Design of Food Blends for the Export Market (R. A. Anderson)

- a. Specific Objective: Continue development and evaluation of new cereal food products with improved nutritional quality.

Progress: Time-temperature studies were carried out with millet to determine processing conditions necessary for lipid enzyme inactivation. Fat acidity values were significantly improved for millet that had been processed for 12 minutes at 200°F. The cooked millet was milled into both high fat and low fat meals by changes in tempering conditions. Functional properties, aside from slightly increased water absorption, changed little except for improved cooking tolerance. Research studies provided a basis for recommending the inclusion of up to 10% nonfat dry milk for wheat-soy-blends utilized in export markets.

Publications:

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BOOKWALTER, G. N. Microwave Processing of Blended Foods. Presented at the Corn Dry Milling Conference, Peoria, Illinois, June 1982.

BOOKWALTER, G. N., H. A. HIGHLAND, AND K. WARNER. Compatibility of Tricalcium Phosphate Addition to Food for Peace Commodities. Presented at the Institute of Food Technologists Annual Meeting, Las Vegas, Nevada, June 1982.

E. NATURAL TOXICANTS AND MICROBIAL TOXINS

1. Trickle Sulfur Dioxide Treatment for On-Farm Drying of Corn at Ambient Temperatures (Cooperative Agreement - Purdue University)

- a. Specific Objective: Study on-farm systems employing sulfur dioxide as mycostatic agent to control microorganisms and minimize quality deterioration during extended periods of corn drying.

Progress: Data resulting from field tests in which the effects of sulfur dioxide (SO₂) addition to high-moisture corn are being evaluated. The study involved a comparison of three different methods of applying the SO₂ to the grain, i.e.: (1) the trickle procedure where 0.12% SO₂ (wt. SO₂/wt. wet corn) is forced up through the grain mass, (2) the reverse trickle where the same

amount of SO₂ is sucked down through the corn by reversing the fan during the treatment, and (3) an auger treatment where SO₂ is injected into the auger during bin loading [0.159% (wt. SO₂/wt. wet corn)]. Additional dosages of 0.05% SO₂ were applied to the trickle-treated corn after 28 and 128 days of storage. After 128 days, the auger-treated bin was visibly molded and an 0.05% SO₂ dose was applied. The control bin showed a high incidence of mold growth in a few days. To date, Purdue has reported on the distribution and loss of SO₂ within the treated bins over the period November 3, 1981, through the winter of 1981-1982. SO₂ content was monitored by a procedure, the water-extractable SO₂ method, developed by Purdue scientists. They observed that the higher moisture corn (26-28%) has a greater affinity and capacity for SO₂ than corn at 14-16% moisture. While evaluation for mold invasion and mycotoxin production is not completed, indications are that the percent of mold-infected kernels in the two "trickle-treated" bins remained near zero through 60 days. The mold-infected corn in the auger-treated bin started to increase slightly after 15 days and had nearly 50% infected kernels after 30 days. On the basis of this study, the auger system appears to be unsuitable for on-farm use.

Report:

ECKHOFF, S. R., J. TUIITE, G. H. FOSTER, M. R. OKOS, and R. A. ANDERSON. Sulfur Dioxide as a Mycocidal Adjunct for Low Temperature Grain Drying. Presented at American Association of Agricultural Engineers meeting, Chicago, Illinois, December 14-17, 1982.

CEREAL SCIENCE AND FOODS LABORATORY

G. E. Inglett, Chief

Research Leaders: F. R. Dintzis and J. S. Wall

A. TECHNOLOGIES FOR FOOD AND FEED USES OF FIELD CROPS

1. Corn Starches--Physical Characteristics and Biological Digestibilities (F. R. Dintzis)

- a. Specific Objective: To complete development of methods for DSC studies of single-kernel corn samples: Evaluate single and double endosperm mutant samples for amylose content and gelatinization behavior.

Progress: Small scale wet-milling procedures were developed that permit isolation of starch from single kernel corn samples in sufficient purity to allow measurement of gelatinization behavior by differential scanning calorimetry (DSC). Samples isolated by these techniques were found to exhibit different DSC characteristics than do starches obtained by commercial wet-milling procedures. This difference in gelatinization behavior was found to be due to the lack of a heating step in drying the single-kernel samples. If single kernel starch samples are heated to 50°C in the presence of water prior to DSC measurement, the gelatinization endotherms are typical of those obtained with commercial starches. The variability in the gelatinization endotherm is a function of temperature, time of heating, and water content. It thus appears that this "annealing" effect has an influence on the crystalline nature of the starch granules. Commercial starches apparently undergo this transformation during the milling and/or drying process and thus cannot strictly be considered as "native" granules. This fact has implications for other aspects of the nature of the starch granule, e.g., susceptibility to enzyme attack.

Publications:

KNUTSON, C. A., J. E. CLUSKEY AND F. R. DINTZIS. Properties of Amylose-Iodine Complexes Prepared in the Presence of Excess Iodine. Carbohydr. Res. 101 (1982):117-128.

KNUTSON, C. A., U. KHOO, J. E. CLUSKEY AND G. E. INGLETT. Variation in Enzyme Digestibility and Gelatinization Behavior of Corn Starch Granule Fractions. Cereal Chem. 59 (1982):512-515.

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CHARALAMBOUS, G. AND G. E. INGLETT. Chemistry of Foods and Beverages: Recent Developments. Academic Press, Inc., New York, NY, pp. 348, 1982. (Conference Proceedings)

2. Methods of Analysis to Facilitate Genetic Improvements of Cereal Grain Protein (J. S. Wall)

- a. Specific Objective: Apply high-performance liquid chromatographic methods to the analysis and characterization of cereal proteins.

Progress: For the first time, reverse-phase high-performance liquid chromatography (RP-HPLC) has been used to characterize cereal proteins. High-resolution separations of albumins, globulins, prolamins, and reduced glutelin polypeptides have been obtained for several cereals using recently-developed porous derivatized silica columns. Resolution equals or exceeds that obtained by any other chromatographic or electrophoretic method for most samples. RP-HPLC has the advantages over other chromatographic and electrophoretic methods of increased speed (ca. 1 hr./run), sensitivity, reproducibility, and ease of quantitation. Since it complements other separation methods, which differentiate proteins only on the bases of size or charge. RP-HPLC promises to be the most significant analytical and preparative technique ever applied to cereal proteins.

A partial listing of the many significant accomplishments achieved through use of this method follows. (1) Wheat varieties can be differentiated and identified through gliadin analysis as an alternative to polyacrylamide gel electrophoresis. (2) Chromosomal control of wheat endosperm proteins has been demonstrated through aneuploid analysis; marker proteins which may be associated with specific agronomic characteristics are thus available. (3) Relationships between different solubility classes (i.e., albumins, globulins, prolamins, and glutelins) have been demonstrated. (4) Homogeneity or heterogeneity of samples has been elucidated. (5) Proteins of interest have been purified on a preparative basis. (6) A method has been found to quickly and positively differentiate tetraploid (durum) and hexaploid (bread) wheats. (7) Gliadin proteins of durum wheats have been identified by RP-HPLC which are excellent positive and negative predictors of pasta quality characteristics. (8) Durum gliadin proteins associated with pasta quality have been isolated by preparative RP-HPLC for subsequent characterization and use in immunochemical tests for durum quality in early generation screening. (9) Methods have been devised for isolation and analysis of several protein classes from individual kernels of cereal grains; the method may be used to determine sample homogeneity/heterogeneity. (10) Demonstration of unusual genotypes, selected through variant phenotypic expression, has been facilitated by RP-HPLC.

- b. Specific Objective: Obtain detailed sequence information on purified endosperm protein fractions from corn, wheat, and other cereals.

Progress: A study comparing the NH₂-terminal sequences of prolamins from teosinte, Tripsacum, sorghum, millet, oats, and rice to those previously determined for corn, wheat, barley, and rye has been completed. The study demonstrates that (a) that significant homology occurs among prolamins in all cereals and (b) that evolutionary relationships can be demonstrated or predicted through sequence analysis.

Sequence analysis of a high-methionine maize glutelin protein of MW ca. 15,000-16,000 daltons, done previously, has been used to identify the NH₂-terminal residue and to confirm the frame-shift of independently-determined nucleotide sequence data, permitting for the first time prediction of the complete amino acid sequence of this protein.

- c. Specific Objective: To further develop isolation methods for obtaining prolamins fractions from corn for continued detailed amino acid sequence information.

Progress: Native and reduced/alkylated zein have for the first time been subjected to reverse-phase high-performance liquid chromatography on a preparative basis. Resolution (15-25 peaks) is similar to that obtained on analytical columns; since in excess of 30 mg may be fractionated within one hour, the method has significant potential for separating purified zeins for sequence analysis in large quantities. Experiments are currently in progress to characterize zein fractions separated by this method in terms of size, isoelectric point, amino acid composition, and degree of homogeneity.

Various extraction procedures were compared for ability to separate alcohol-soluble fractions of reduced glutelin in order to establish protein relationships. The most effective procedure for separation of proline-rich and methionine-rich fractions was by solubility in water; the former component is soluble in this media. The various fractions were compared by isoelectric focusing in polyacrylamide gel, electrophoresis in pH 3.5 aluminum lactate buffer and pH 8.7 sodium dodecyl sulfate buffer and amino acid analysis.

- d. Specific Objective: Study relationships between prolamines in various genetic backgrounds of corn and sorghum grains.

Progress: A polyacrylamide 2-dimensional electrophoresis system consisting of isoelectric focusing (pH 6-8) in the first direction and aluminum lactate buffer (pH 3.5) in the second direction has

been employed to attain separation of zeins extracted from various genotypes of corn. The method has been improved by use of silver staining to detect the various proteins in the electrophoretic pattern. This staining system has permitted reduction in the size of the sample applied with increase in resolution of spots and also differentiated some proteins by spot color. It was established that all of the different inbreds examined had different electrophoretic patterns. The extent of differences varied; the inbreds derived from different corn breeding groups had the greatest variation. The pattern of proteins could be used to identify the inbred line. Hybrids obtained by crossing 2 inbreds had patterns containing all protein derived from both parents. Zeins of new inbreds obtained by crossing established F_2 progeny had some protein spots derived from either parent but did not have all spots present in both parents. Examination of zeins from the inbred W64A in which mutant genes such as O2 and fl2 were introduced indicated that these mutant influenced production of specific zeins.

We have also demonstrated by reverse-phase HPLC the additive nature (with the expected 2/1 ratio) of zein expression by maternal and paternal parents in corn hybrids. In addition, we have shown that significant differences occur among inbreds and among hybrids, suggesting that RP-HPLC will be useful for identification of inbreds and hybrids, for establishment of genetic relationships, and for aneuploid analysis. Reverse phase HPLC has been also used to separate alcohol-soluble fractions of reduced glutelin (ASG). The elution relative position of major components of the water-soluble ASG, water-insoluble ASG and zein fractions differed, further confirming differences in these solubility classes of proteins.

- e. Specific Objectives: Establish nature of protein changes in high-protein and high lysine wheats.

Progress: Different strains of high protein and high lysine wheats were obtained from the University of Nebraska. The grains were analyzed for protein and lysine content. Proteins were extracted from the flours derived from the kernels using a buffer containing acetic acid-cetyl trimethyl ammonium bromide-urea. The proteins were separated according to class by gel filtration chromatography. For this purpose, new TSK high speed columns were employed. The amounts and amino acid composition of the different protein fractions were determined. This information is being compiled to relate changes in protein composition to protein and lysine levels of new grain types.

- f. Specific Objective: Determine the effect of germination of oats on protein fractions and amino acid composition.

Progress: Terra oats (hulless) were germinated at 20 and 25°C in the dark and at 23°C in room light for 8 days. Dry matter loss was

5 to 6% after 3 days of germination but increased to 17 to 20% after 8 days. Nitrogen content of germinated oats was higher after 3 days of germination as a result of dry matter loss. Proteins in germinated oats were fractioned by water, 1 M sodium chloride, 70% ethanol, and 0.005 N sodium hydroxide plus dithiothreitol sequentially. There was an increase in water-soluble N and a decrease in salt-soluble N as germination proceeded. The increase in water-soluble N was due to an increase in nonprotein N rather than albumin. Lysine, expressed g/16 g N, was higher in oats germinated for 6 and 8 days compared with ungerminated oats.

- g. Specific Objective: To determine if wheat proteins yield peptides upon digestion that have (opioid) exorphin activity.

Progress: A collaborative project (see A.7.) has been initiated with Cornell University Medical School - New York Hospital Medical Center to investigate a possibility that the high coincidence of schizophrenia in patients suffering from celiac disease may be due to a biochemical disorder. Wheat proteins were isolated from the grain and separated by solubility and chromatography into various gliadin and glutenin fractions. These protein fractions were digested with pepsin to yield mixtures of peptides which were sent to Dr. R. Lieberman at Cornell University Medical School for assay for exorphin activity. The assay determined competitive binding by the peptides to sites in rat brain tissue where morphine or its derivatives are bound. The procedure involved incubating peptides and ¹⁴C labeled dehydromorphine with rat tissue. Initial results indicated high activity in peptides derived from certain gliadin proteins.

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ESEN, A., J. A. BIETZ, J. W. PAULIS, AND J. S. WALL. Tandem Repeats in the N-Terminal Sequence of a Proline-Rich Protein from Corn Endosperm. Nature 296 (1982):678.

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BIETZ, J. A., L. A. COBB, AND J. S. WALL. Wheat Varietal Identification and Genetic Analysis by Reverse-Phase HPLC of Proteins Extracted from Single Kernels. Presented at the 67th Annual Meeting, Assoc. Cereal Chemists, San Antonio, TX, October 24-28, 1982. *Cereal Foods World* 27(9) (1982):470.

BIETZ, J. A. Analysis of Cereal Proteins by HPLC. Presented at the 29th Annual Soft Wheat Quality Laboratory Research Review Conference, Wooster, Ohio, April 15, 1982.

WALL, J. S., D. A. FEY, AND J. W. PAULIS. Improved Two-Dimensional Electrophoretic Separation of Zein Proteins. Application to Identification of Zein Genotypes. Presented at the 67th Annual Meeting, Association Cereal Chemists, San Antonio, Texas, October 24-28, 1982. *Cereal Foods World* 27(9) (1982):449-450.

WU, Y. V., J. W. PAULIS, K. R. SEXSON, AND J. S. WALL. Conformation of Corn Zein and Glutelin Fractions with Unusual Amino Acid Sequence. Presented at the Federation of American Societies for Experimental Biology 66th Annual Meeting, New Orleans, Louisiana, April 15-23, 1982.

3. Epoxides from Lipid Hydroperoxides and Their Interactions in Cereal and Oilseed Foods (H. W. Gardner)

- a. Specific Objective: Explore novel ways of degrading hydroperoxides to obtain new information on mechanisms of rancidity development.

Progress: The mechanism of lipid hydroperoxide decomposition by acids in protic solvent was fully characterized. The mechanism was tested to synthesize epoxyhydroxyene and trihydroxyene fatty acids which are products of certain biological systems.

The mechanism of lipid-cysteine adduct formation from the free radical reaction of linoleic acid hydroperoxides and cysteine also was investigated. This reaction is thought to be significant in the lipid to protein crosslinking observed in peroxidizing food systems. Four different diastereomeric compounds were isolated and characterized, and these compounds proved to be unstable intermediates that defined the mechanism of the reaction.

Publications:

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GARDNER, H. W., C. G. CRAWFORD AND J. T. MACGREGOR. Epoxide Fatty Methyl Esters Derived from Homolysis of Linoleic Acid Hydroperoxides are not Mutagenic by Ames Test. Food Chem. Toxicol. In press.

VAN OS, C. P. A., J. F. G. VLIEGENTHART, C. G. CRAWFORD AND H. W. GARDNER. Structural Analysis of Diastereomeric Methyl 9-Hydroxy-trans-12,13-Epoxy-10-trans-Octadecenoates. Biochim. Biophys. Acta 713 (1982):173-176.

Other Reports:

GARDNER, H. W. AND E. C. NELSON. Acid-Catalyzed Decomposition of Linoleic Acid Hydroperoxides. Presented at the AOCS Meeting, Toronto, Canada, May 3-6, 1982.

GARDNER, H. W. Effects of Lipid Hydroperoxides on Food Components. Presented at the ACS Meeting, Kansas City, KS, September 11-18, 1982.

GARDNER, H. W. The Chemistry of Lipid Hydroperoxides: Does the Chemical Decomposition Act as a Model for Biochemical Degradation? Presented at the AOCS Meeting, Toronto, Canada, May 3-6, 1982.

4. Molecular Structure of Maillard-Type Browning Reaction Products (H. B. Sinclair)

- a. Specific Objective: A new route to the Amadori compound, glycinofructose has been devised and will be carried out.

Progress: Conversion of fructose into 1-O-methanesulfonyl-2,3:4,5-di-O-isopropylidene-D-fructose was carried out in a two-step reaction sequence. Displacement of the methanesulfonyl group with amines and amino acid esters to yield an Amadori type compound was unable to be accomplished. A wide variation of reaction conditions, such as solvent, temperature, and amine structure, was tried. A moderate yield of the corresponding 1-azido-di-isopropylidene fructose was obtained by extended reaction (4-6 days) of the methanesulfonyl derivative in boiling dimethylformamide. Catalytic hydrogenation of the azide group with Pd/C to give an amino group could not be carried out on repeated attempts. Chemical reduction using lithium metal in boiling ethanol produced a very low yield of the 1-amino-1-deoxy-2,3:4,5-di-O-isopropylidene-D-fructose. With methyl iodide this amine could not be alkylated.

Although minor difficulties were expected in this approach, the low yields and general experimental problems warrant that this approach to Amadori compounds be discontinued.

- b. Specific Objective: To investigate the reactivity of isomaltol with primary, secondary amines, amino acids, and aldehydes which are found in foods.

Progress: The isolation of glucosyl- and galactosylisomaltol from the nonenzymatic browning of maltose and lactose with secondary amino acids (DL-pipecolic acid, L-(-)-proline, sarcosine) demonstrated new chemical pathways. It was found that ion exchange resin (H⁺ form) column chromatography was effective in isolating glucosyl- and galactosylisomaltol at reaction yield levels of 6 and 7%, respectively. It was discovered that galactosylisomaltol also resulted from the interaction of lactose with the primary amino acids, alanine and glycine at less than 3% yield. A condensation product was isolated from the interaction of isomaltol and L-(-)-proline, and a low molecular weight polymeric product from the reaction of isomaltol with glycine. This low molecular weight polymeric product was found to be a self-condensation product of isomaltol, and it is water insoluble. Alkali metal (sodium) complexes of isomaltol and maltol were isolated and characterized.

- c. Specific Objective: To study the preparation and browning reactions of model Amadori compounds in order to evaluate non-enzymatic browning in foods.

Progress: It has been shown that 3-O-methyl-D-glucose reacts with piperidine and with di-N-butylamine to form in each case a pair of Amadori compounds. Similarly two Amadori compounds have been detected in the reaction of piperidine with glucose although only one has been reported earlier. The reaction of 3-O-methyl-D-glucose with morpholine, however, produces only one Amadori compound. This compound has been isolated as its di-p-nitrobenzoate. Its exact structure awaits the outcome of n.m.r. spectroscopy. The principal Amadori compound from the reaction of 3-O-methyl-D-glucose with piperidine has also been isolated as its di-p-nitrobenzoates.

- d. Specific Objective: Conduct Maillard (browning) reactions with model compounds to determine the influence of mineral cations on melanoidin formation under different conditions of pH, temperature, and concentration. Isolate melanoidins prepared from various carbohydrates and amino compounds and investigate their composition, molecular structure, and mineral-binding ability for the purpose of obtaining information that will be helpful in relating the Maillard reaction in foods to food safety and quality.

Progress: By means of spectrophotometry and fluorometry, the presence of Cu²⁺ in a glucose (1.25M)-glycine (0.66M) system at 50°C was shown to influence the rate of browning. At pH 3 an increase in Cu²⁺ concentration led to a faster rate of browning during the initial stage of reaction (~300 hours); and beyond this

stage, the production of melanoidin was always greatest in those reaction mixtures containing the highest concentration of copper. Maximum copper concentration employed in these studies was 100 ppm. Not only did Cu^{2+} catalyze the formation of melanoidin from premelanoidins (unsaturated precursors), but it also catalyzed the formation of premelanoidins. At pH 6.2 (initial) Cu^{2+} enhanced the rate of browning during the initial stage of reaction (~100 hours); however, in later stages the copper often appeared to depress the rate of formation such that the copper-free system would have the highest yield of melanoidin. Interpretation of the data on the pH 6.2 reaction was made difficult by the absence of pH control; over a period of 900 hours, the pH dropped from 6.2 to 4.4. Future work at or about pH 6 will be conducted with automatic control of pH.

Maillard reactions conducted at pH 3 were relatively slow and led to the isolation of highly insoluble melanoidins whose nitrogen content varied slightly according to the molar ratio of glucose to glycine in the initial reaction mixture. Rates of formation were a function of both the concentration of glucose and the concentration of glycine. Reaction at pH 6.2 (initial) was much faster than at pH 3 and produced a water-soluble melanoidin (average molecular weight of about 50,000) that was isolated as a brown solid by means of dialysis and lyophilization.

Cu^{2+} ions were shown to be strongly complexed by water-insoluble melanoidin, even in moderately acidic systems (pH 3). In the pH range of 4-6 these metal cations were found to be unstable in closed, oxygen-free systems. In the absence of O_2 , Cu^{2+} was slowly reduced to metallic copper. However, in oxygenated systems no metallic copper was formed.

Comparison of browning reactions of various carbohydrates with glycine were made at both pH 3 and pH 6.2 (initial). For the pH 3 runs, reactivity decreased in the following order: 5-hydroxymethylfurfural >> glyoxal >> (glucose, 3-O-methylglucose, fructose). At pH 6.2 (initial) the order was somewhat similar: 5-hydroxymethylfurfural > glyoxal >>> 3-O-methylglucose > glucose > fructose.

Publications:

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GOODWIN, J. C. Isolation of 3-O- α -D-glucosyl- and 3-O- β -D-galactopyranosyloxy-2-furyl Methyl Ketones from Nonenzymatic Browning of Maltose and Lactose with Secondary Amino Acids. (Accepted by Carbohydr. Res. on June 16, 1982).

GOODWIN, J. C. AND J. E. HODGE. Sweetness and Bitterness of Some Aliphatic α,ω -glycol-D-glucopyranosides. NOPABCCHE Abstr. of Papers p. 18. 1982 (Abstract).

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GOODWIN, J. C. Sodium Complexes of Isomaltol and Maltol. For Carbohydr. Res. In preparation.

INGLETT, G. E. Maize Chemistry and Technology: Recent Progress. Academic Press, Inc., New York, NY, 1982. (Conference Proceedings)

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RENDLEMAN, J. A. AND C. A. GROBE. Cereal Complexes: Binding of Zinc by Bran and Components of Bran. Cereal Chem. 59 (1982):310-317.

SINCLAIR, HENRY B. Reaction Pathway in the Base Transformation of 2,4-O-benzylidene-1,6-di-O-p-tolyl-sulfonyl-D-glucitol into 1,3-anhydro-2,4-O-benzylidene-D-glucitol. (Accepted for publication in Carbohydr. Res.).

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GOODWIN, J. C. Isolation of 3-O- α -Glucopyranosyloxy-2-furyl methyl ketones from Nonenzymatic Browning of Maltose and Lactose with Secondary Amino Acids. Presented at the 184th National Meeting of the American Chemical Society, Kansas City, MO, September 12-18, 1982.

INGLETT, G. E. The Potential for the Modification of Carbohydrate Foods. In "The Role of Food Product Development in Implementing Dietary Guidelines," G. E. Livingston, R. J. Moshy and C. M. Chang, eds., Food and Nutrition Press, Inc., Westport, CT, pp. 212, 1982. (Conference Proceedings)

LINEBACK, D. R. AND G. E. INGLET. Food Carbohydrates. Proceedings of the Pre-IFT Symposium, Atlanta, Georgia, June 5-6, 1981. Avi Publishing Co., Inc., Westport, CT, 1982. (Conference Proceedings)

5. Basic Research on Metabolic Regulation of Plant Tissues (G. E. Inglett)

- a. Specific Objective: Determine the effects of exogenously applied cytokinins and related phytohormones on the seed quality of Zea mays mutants containing amylose-extender (ae) and waxy (wx) loci along with related maize seeds.

Progress: Preliminary studies on the culturing of Zea mays mutants containing amylose-extender (ae) and waxy loci along with its background material and a hybrid involved collecting the mature seeds from plants which had been exogeneously treated with phytohormones during growth in statistically-designed experiments at NRRC. Other maize seeds derived from plant treatments with triacontanol (also with and without indoleacetic acid) were obtained from a cooperating scientist. Another cooperator provided white maize seeds obtained from treated growing plants with 4 levels of 1,1-dimethylpiperidium chloride. All these maize samples are in various stages of chemical and biological evaluation. Sufficient data are not available at this time to indicate the degree of influence that the bioregulators had on seed quality. The cooperators provided seeds which came from treated maize plants that appeared to give increased yields of grain but of unknown quality. The quality and composition of the maize mutant seeds obtained from the NRRC plots are being determined although the level of cytokinin treatment appeared too high which greatly reduced seed development. Furthermore, an interaction of cytokinin with another phytohormone appeared to drastically interfere with ear development and seed set.

- b. Specific Objective: Elucidate indigenous actions of phytohormones in plants including Zea mays seeds involving imbibition, germination, and dormancy.

Progress: A simple one-step procedure was developed to synthesize traumatin, a plant wound hormone, from 13-hydroperoxy-cis-9, trans-11-octadecadienoic acid. The cleavage reaction is catalyzed by a Lewis acid in aprotic solvent. This reaction should give a clue to the mechanism of the biosynthetic route which catalyzes the same cleavage by the enzyme, hydroperoxide lyase. Only preliminary

experiments are underway on the actions of phytohormones in Zea mays seeds involving imbibition and germination related to dormancy.

6. Isolation, Purification, and Characterization of Corn, Alcohol-Soluble Proteins (Cooperative Agreement - Virginia Polytechnic Institute and State University)

- a. Specific Objective: Isolate alcohol-soluble corn proteins, cleave them by specific chemical and enzymic processes and separate peptides for amino acid sequencing.

Progress: Further modifications of ion-exchange procedures for improved separation of alcohol-soluble corn proteins were conducted in order to provide samples for NRRC sequence studies. Alcohol-soluble reduced glutelins (ASG) were fractionated on Sulfoethyl-Sephadex with a salt gradient. After separation from water-soluble components the methionine-rich fraction was resolved on a chromatofocusing column. The eluted fractions were still heterogenous by isoelectricfocusing on gels. Zein components were prepared by chromatography on CM Sepharose and purified by preparative isoelectric focusing. To prepare peptides for sequencing zein, the protein was cleaved chemically and with proteolytic enzymes. Extensive cleavage of zein with CNBr occurred in trifluoroacetic acid but only 2 components resulted when the reaction occurred in acetic acid. Use of 0.5% sodium dodecyl sulfate facilitated cleavage of zein with chymotrypsin into fragments ranging from 3000 to 14,000 which were separated by gel filtration.

- b. Specific Objective: Isolate RNA coding for water-soluble, alcohol-soluble corn proteins to permit preparation and cloning of DNA.

Progress: Water-soluble ASG was bound to sepharose columns and used to isolate antibodies specific for this protein from serum from rabbits inoculated with the protein. Protein bodies were isolated from homogenates of immature corn and extracted to yield proteins identified by electrophoresis as water-soluble ASG. Other researchers had not found this protein in isolated protein bodies.

Publication:

ESEN, A., J. A. BIETZ, J. W. PAULIS AND J. S. WALL. Tandem Repeats in the N-Terminal Sequence of a Proline-Rich Protein from Corn Endosperm. Nature 296 (1982):678-679.

7. Exorphin Activity of Separated Peptides of Wheat (Cooperative Agreement - New York Hospital-Cornell Medical Center)

- a. Specific Objective: Assay for opioid (exorphinyactivity) peptides derived from wheat protein to establish which peptides are most active.

Progress: A series of peptic hydrolyzates of 16 selected purified fractions of wheat proteins including gliadins, glutenins, and globulins was prepared by F. Huebner (NRRC) and submitted to Dr. K. Lieberman (Cornell University Medical School) for assay for opioid activity. Of the protein hydrolyzates those obtained from α -gliadins appeared to have the greatest activity. Assay for opioid activity was based on competitive binding of the peptides with rat brain tissue in the presence of ^{14}C labeled dehydromorphine which binds to opioid analgesic activity sites. A series of peptide fractions isolated from hydrolyzates of active α -gliadin are currently being assayed for opioid activity to establish the nature of peptides exhibiting such activity.

B. BIOMATERIALS SCIENCE

1. Enzymatic Conversion of Cellulose to Sugars for Alcohol Fermentations (F. R. Dintzis)

- a. Specific Objective: Characterize factors that limit efficiency of enzymatic conversion of cellulose by "complete" cellulase systems to sugars for alcohol fermentation.

Progress: Details of a separation method have been completed for obtaining from the complex of Trichoderma reesei, an isolate that disrupts or unravels filter paper fibers without detectable hydrolysis of the substrate paper. SEM evidence shows the appearance of a disrupted α -cellulose filter paper fibers is similar to the appearance of filter paper treated with Fenton's reagent (H_2O_2 and Fe^{+2}). However, our isolate requires Fe^{+3} and the rate of filter paper disruption is faster when the isolate is used than when 0.04% H_2O_2 + 24 μg Fe^{+2} /ml is used. We consider the isolate obtained from Trichoderma reesei to be enzyme free because it is of low nitrogen content, it elutes from the column with low molecular weight materials such as sugars and buffer salts, and the methanol soluble portion of the lyophilized isolate exhibits activity when returned to aqueous solution. The isolate demonstrates heat stability as indicated by the observation that activity is retained after it has been heated in boiling water for 20 minutes.

2. Energy-Saving Methods for Recovery of Usable Protein from Alcohol or Methane Fermentation Media (J. S. Wall)

See Northern Agricultural Energy Center, A.4.

C. CHEMICAL RESIDUES AND ADDITIVES IN FOOD AND FEED

1. Effect of Environmental Contaminants on Cereal Foods and Feeds (W. J. Garcia)

- a. Specific Objective: Complete work on corn and wheat grains grown under conditions of different levels of ambient ozone.

Progress: Study was completed on whole kernel corn derived from field-grown plants that had been exposed to ambient ozone at chronic concentration levels (0.02 to 0.15 ppm O₃). Biochemical changes in the stressed grain are manifested best in trace element and methionine concentration changes, and they appear to be directly connected because of similar effects. Relative increases in methionine content were similar in magnitude to increases observed for copper, iron, and zinc with copper providing the closest correlation. However, responses to the same levels of ozone were different for different corn varieties.

- b. Specific Objective: Continue work on a cooperative project with the ARS Human Nutrition Research Center (HNRC), Grand Forks, ND, involving bioavailability of zinc and include other minerals in prepared food products by adding other labeled radioactive minerals.

Progress: Food products from corn endosperm labeled with low levels of zinc-65, which were prepared at NRRC for subsequent use in human zinc bioavailability studies at HNRC, were successfully used to determine the effects of browned and unbrowned corn products on the absorption of zinc, iron, and copper.

Publications:

CARLSON, K. D., R. L. CUNNINGHAM, W. J. GARCIA, M. O. BAGBY AND W. F. KWOLEK. Performance and Trace Metal Content of Crambe and Kenaf Grown on Sewage Sludge-Treated Stripmine Land. Environ. Pollut. (Series A) 29 (1982):145-161.

GARCIA, W. G., J. F. CAVINS, G. E. INGLETT AND A. S. HEAGLE. Quality of Corn Grain from Plants Exposed to Chronic Levels of Ozone. In preparation for Cereal Chem.

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D. FOOD COMPOSITION AND IMPROVEMENT

1. Action of Human Digestive System Upon Cereal Grain Fiber Sources and Related Foods (F. R. Dintzis)

- a. Specific Objective: Examine effects of passage through the human digestive system upon wheat brans, dry milled corn bran, and soy hulls.

Progress: Additional data on Eagle and Durum wheat brans corroborate the effects seen previously that xylose components of brans are more susceptible to digestion in the human G.I. tract than are cellulose and arabinose containing polymers. We continue to observe that small particles of retrieved bran pericarp have different neutral sugar composition ratios that do the larger particles. For dry milled corn bran, the neutral sugar ratios of the particles retrieved on a 40-mesh are about those of the starting 18 to 30-mesh distribution which indicates little digestion of the larger particles. However, corn bran particles retrieved on an 80-mesh screen do have altered composition ratios which indicates they have undergone some attack in the digestive system.

- b. Specific Objective: To improve methods of dietary fiber analysis using sophisticated methods.

Progress: Initial work shows that the loss of uronic acid obtained under conditions commonly used to hydrolyze plant tissues is only partly caused by decarboxylation. Yields of up to 40% uronic acids can be obtained from pectin hydrolyzates provided proper hydrolysis techniques are used. Use of Seaman hydrolysis methods, i.e., initial contact of substrate with concentrated sulfuric acid, works well but requires complete removal of sulfate ion to prevent destruction of sugars during the acetylation step. Attempts to use methanol in a solvent separation method to remove sugars from sulfate ion were not successful. Use of barium or lead carbonate to remove sulfate ion as a precipitate was determined to be a better method.

- c. Specific Objective: Cooperate with the ARS Human Nutrition Research Center to determine effects of fiber in the diet.

Progress: Phytic acid contents have been made on a large number of whole diets fed to human volunteers. The HPLC method developed by Graf and Dintzis was used in the measurements in which the phytate content of these high fat, high protein diets varied from about 0.25% for diets containing hard red spring wheat bran in the daily portion to about 0.04-0.02% for low fiber basal diets.

- d. Specific Objective: Study iron binding properties of wheat brans and submit manuscript for publication.

Progress: This study is nearly completed and awaits a proper determination of residual phytate in brans exposed to iron. About 25% of the ferric iron bound by wheat brans during in vitro measurements simulating human gastric conditions can be attributed to phytate remaining in the pericarp. However, other mechanisms of binding are required to explain the remainder of iron binding. It was observed that at short times of 2 to 4 hours, the amount of ferric iron bound was about 3 times greater than the binding of ferrous iron. At long times, 20-30 hours, the binding of the two forms of iron by bran pericarp was similar.

- e. Specific Objective: Improve HPLC method for phytic acid determination in general food samples and prepare labeled phytic acid for interaction studies.

Progress: An improved HPLC method for phytic acid determination in general food samples has been developed and applied to a variety of foods and whole diets. A technique for preparing labelled phytic acid from germinating wheat seeds has been established and successfully used to obtain tritium and phosphorus 32 labeled phytate.

- f. Specific Objective: Examine mineral binding properties of wheat brans that have passed through the human digestive system.

Progress: Initial comparison of the Cu, Fe, Zn, and Ca content of bran pericarp before it is baked in bread and after it is retrieved from human fecal matter, indicates a possible increase in iron content and a definite increase in Ca content. Atomic absorption is used to measure mineral contents of the brans.

Publications:

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FERMENTATION LABORATORY

C. W. Hesseltine, Chief

Research Leaders: R. W. Detroy, M. D. Grove, C. P. Kurtzman,
O. L. Shotwell, and M. E. Slodki

A. PHYSIOLOGICAL AND BIOCHEMICAL TECHNOLOGY TO IMPROVE CROP PRODUCTION

1. Polysaccharides in Specific Associations of Nitrogen-Fixing Microbes with Plants (M. E. Slodki)

- a. Specific Objective: Search for factors involved in preferential nodulation of soybean varieties by strains of Rhizobium japonicum.

Progress: Three lines of evidence confirm the presence in seeds of all soybean varieties tested, including the "lectinless" and "non-nod," of a new lectin activity having multivalent binding specificity toward 4-O-methyl-D-glucurono-L-rhamman:precipitin bands formed in Ouchterlony double diffusion gels; precipitin ring formation in solution; and polar agglutination of washed cells. Dissolution of the bands by 4-O-methyl-D-glucuronic acid, D-glucuronic acid and their methyl glycosides, but not by any other sugars or uronic acids, demonstrated the binding specificity. D-Glucuronic acid also inhibits formation of characteristic head-to-head, rosette assemblages of washed cells. In contrast to the nonspecific side-by-side agglutination, the rosettes reform soon after disaggregation by sonic treatment. Classical, galactose-specific, soybean lectin does not interact with the polysaccharide; 7S and 11S soy protein fractions are also inactive.

- b. Specific Objective: Continue structural investigations of polysaccharides from competitive and less-competitive strains.

Progress: All attempts to obtain phage-associated depolymerases active on 4-O-methylglucurono-rhamnan were unsuccessful. By soil enrichment culture techniques, however, a Bacillus was isolated that produces a relatively stable depolymerase. Viscosities of the extracellular polysaccharide are rapidly diminished. No free monosaccharides are released; the sole product is an oligosaccharide (by HPLC), which likely represents the tetrasaccharide repeat unit of the polysaccharide. In addition to providing a potential hapten inhibitor, the enzyme could be used to "fingerprint" related polysaccharides produced by rhizobial microsymbionts of temperate and tropical legumes.

- c. Specific Objective: Structural investigations of capsular polysaccharides from tropical rhizobia.

Progress: Completion of structural studies on the capsular polysaccharide (CPS) from microsymbionts of Acacia decurrens was facilitated by the finding that extensive hydrolysis of the permethylated CPS degraded rhamnosyl groups. Mild formolysis established the proportion of rhamnosyl groups and indicated that the D-glucosyl branch points occur as a β -(1 \rightarrow 3)-linked disaccharide. A CPS from a microsymbiont of peanut is similar in composition to the CPS from rhizobia associated with A. decurrens except that D-galacturonic acid is replaced by a hexose bearing pyruvic acetal. Many microsymbionts of tropical legumes have PS/CPS that resemble those of R. japonicum; many others, however, have quite different compositions. In cooperation with H. Keyser, ARS Agricultural Research Center, Beltsville, MD, nodulation and serotyping correlations are being made for those involved in nodulation of soybeans. Component analyses have been done on CPS from tropical rhizobia that nodulate wide spectra of host legumes.

d. Specific Objective: Improve methodologies.

Progress: Conditions for the graded hydrolysis of R. japonicum B-4421 4-O-methyl-glucuronorhamnan in 0.5 N aqueous trifluoroacetic acid (TFA) at 70°C were established. Thin-layer chromatography and gas liquid chromatography analyses showed that molecular weights can be tailored for a wide range of oligosaccharides varying in average degree of polymerization from 8 to 40. Apparent discrepancies in polysaccharide compositions, as judged after hydrolysis and gas liquid chromatography analysis, were investigated. Monosaccharide stabilities in aqueous TFA and Stellner reagent were checked, as were detector (F.I.D.) responses to alditol acetates and per-O-acetylaldononitriles. Greatest stability was found for aqueous TFA; Stellner reagent degrading D-glucose and D-mannose at rates exceeding that for D-galactose. F.I.D. correction factors allowed accurate evaluation of hydrolyzates from PS B-4421 (see b. above).

Investigation of phenacyl ester formation and subsequent reduction for acidic polysaccharides nears completion. Steric hindrance of esterification was noted for polysaccharides known to be so hindered, whereas accessible uronic acid residues were completely converted to carbinols in polysaccharides B-4421, B-1459, 1B3, and R.j. 123.

Preparation of an affinity column of Sephadex bearing D-glucuronic acid is anticipated, and a four-step synthesis of a blocked precursor, 6-(benzyloxycarbonylamino)hexyl 2,3,4-tri-O-acetyl-D-glucuronic acid methyl ester, was accomplished. A synthesis scheme for the 4-O-methyl analog has been devised, and the initial two steps undertaken. This type of affinity column should be useful in isolation of the newly discovered soybean lectin.

Publications:

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2. Physiology of Nitrogen-Fixing Blue-Green Algal and Rhizobial Symbioses with Plants (J. W. Newton)

- a. Specific Objective: Study unique polypeptides of the Azolla blue-green algal nitrogen-fixing symbiosis.

Progress: Cyanophycin granule polypeptide (CGP) has been identified as a nitrogenous component present in Azolla caroliniana plants which are fixing nitrogen. This polymer, which is multiarginine-polyaspartic acid (1:1), is known to be a major constituent of akinetes of blue-green algae that accumulate as senescent cells in older cultures. This finding indicates that a significant fraction of Azolla nitrogen is CGP formed as the leaf is colonized by the

algae in nitrogen-fixing plants. Because of the agronomic importance of this finding, further studies were undertaken on the formation and breakdown of this polymer in nitrogen-fixing plants.

^{14}C -labeled CPG rapidly breaks down (10% in 24 hours) in paddy (insoluble fraction) and other soils to give $^{14}\text{CO}_2$ even though it is generally resistant to common proteolytic enzymes. The various soils were comparable in activity and were inactivated by autoclaving.

- b. Specific Objective: Isolation and study of blue-green algae associated with Azolla species.

Progress: Strains of blue-green algae (Nostoc sp.) which are capable of simultaneously forming akinetes and fixing nitrogen have been isolated from paddy soils in which Azolla is abundant. These strains form cyanophycin granule polypeptide (CGP), the copolymer of arginine and aspartate also found in Azolla leaves colonized by blue-green algae. When the algae are placed under growth-limiting conditions by phosphate depletion, an enhancement of nitrogenase activity and decline of H_2 evolution occurs coincident with extensive akinete formation and production of CGP by the cells. These studies indicate that CGP may play an important role in nitrogen deposition and turnover in soils which contain blue-green algae in abundance.

A sample of slow-growing Azolla leaf tissue has been successfully restarted in culture. The callus grows much better on a complex microbiological-type of medium than on the usual plant tissue culture media. Plant growth hormones kill the tissue.

- c. Specific Objective: Study the pleomorphic cells of acetylene-reducing rhizobia to determine their relationship to the activity.

Progress: Cowpea-type Rhizobium 32H1, R. japonicum USDA 26 and 110 grown on a glutamate-mannitol-gluconate agar medium exhibited increases in AR^+ pleomorphic cells coincident with their acetylene-reducing (AR^+) activity. In contrast, strain 10 was consistently AR^- , even though it also could grow and yield pleomorphic cells under various conditions. Cell-division inhibitors (nalidixic acid and novobiocin) increased pleomorphy also, but generally inhibited AR^+ activity. The study concludes that pleomorphic cells derived in different ways are not all AR^+ and that formation of a specific pleomorph (perhaps involving changes in permeability of the cell envelopes) is necessary to obtain optimal AR^+ activity.

- d. Specific Objective: Develop conjugation systems that will transfer acetylene-reducing activity among auxotrophs of R. japonicum and cowpea-type strains.

Progress: Tan-colored mutants, which retain the capacity to reduce acetylene, were selected and isolated from R. japonicum L-259 after growth in a glutamate-limited medium supplemented with tryptophan. The mutants catabolized tryptophan, producing orange fermentation broths containing extracellular indolepyruvic and indoleacetic acids. However, tryptophan was an incomplete nitrogen source for mutant growth and did not stimulate acetylene reduction. Parental strain L-259 produced colorless indoleacetic acid alone when tryptophan was added to the growth medium. AR mutants, which yield such defined metabolic products, will be used as donor strains of nitrogen fixation genes that would be expressed symbiotically.

Publications:

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NEWTON, J. W. AND D. D. TYLER. Formation and Breakdown of Azolla Polypeptides in Paddy Soils. Proceedings International Workshop on Practical Applications of Azolla for Rice Production. Mayaguez, Puerto Rico, November 17-19, 1982. In press.

Other Reports:

KANESHIRO, T. AND M. E. SLODKI. Metabolic Mutants (Tryptophan) of an Acetylene-Reducing Rhizobium japonicum. Plant Physiol. Suppl. 69(4) (1982):110.

NEWTON, J. AND D. D. TYLER. Formation and Breakdown of Azolla Polypeptides in Paddy Soils. In Abstracts of Practical Applications of Azolla for Rice Production. International Workshop. University of Puerto Rico, Mayaguez, November 17-19, 1982. p. 15.

3. Nitrogen Contribution of Azolla spp. in Aquatic Farming Systems (Cooperative Agreement - University of Hawaii)

a. Specific Objective: Develop methods for Azolla maintenance.

Progress: A key to the seven known species of Azolla has been prepared using leaf and rhizome morphology data obtained from

scanning electron micrographs. Relative growth rates (RGR) of seven lines or species of Azolla were calculated from weekly growth measurements made over 1 year at Hangzhou, China. Solar radiation, relative humidity, and temperature of water and air were also measured. During spring and fall, 79-92% of the variation in the RGRs of A. caroliniana, A. filiculoides, A. mexicana, A. pinnata, and A. rubra was accounted for in a linear model by minimum water temperature and day length. Significant R^2 values for A. microphylla and A. nilotica were obtained when solar radiation was also included in the model. A. rubra could not tolerate the very warm humid weather of summer. For summer, 74-91% of the variation in RGR of the six species was accounted for in a linear model by minimum water temperature, solar radiation, and relative humidity. The regression models have the potential to predict relative growth rates of the various species at locations where climatic data are available. The RGR of A. microphylla in Hawaii was 0.26 g g⁻¹ day⁻¹ and was 20% greater than that of A. pinnata. In 25% shade, RGRs were 0.17 for both species. In 66% shade, A. pinnata had twice the fresh weight of A. microphylla. The RGR of a mixture of A. pinnata and A. microphylla was 17% greater than that of either species grown alone. The use of species with shade tolerance or in mixtures may increase the quantity of N fixed by Azolla grown as an intercrop with rice or taro. A collection of Azolla sp. obtained worldwide is maintained at the University of Hawaii for distribution to interested users.

- b. Specific Objective: Study nutrient requirements of Azolla, with emphasis on phosphorus.

Progress: The nitrogen contributions of A. caroliniana, A. filiculoides, A. pinnata, A. microphylla, and A. rubra to paddy rice were estimated in 26 m² field plots. Nitrogen yields ranged from a low of 45 kg ha⁻¹ for intercropped A. rubra after 3 weeks' growth to a high of 230 kg ha⁻¹ for monocropped (3 weeks) plus intercropped (3 weeks) A. filiculoides. Rice yield ranged from 4.7 (0 nitrogen) to 6.1 metric tons for the best Azolla treatment. With some soils, phosphorus addition is necessary for optimum use of Azolla as a green manure.

- c. Specific Objective: Determine effects of microbial antagonists on N₂ fixation in paddies.

Progress: Several species of green algae have been isolated which inhibit growth of blue-green algae. These strains are in the collection of the Agronomy and Soil Science Department, University of Hawaii, Manoa.

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LUMPKIN, T. A. China's Organic Fertilizers--A Threatened Tradition. In Agriculture and Rural Development in China Today, Edited by R. Barker, Westview Press, Boulder, Colorado. In press. 1982.

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LUMPKIN, T. A., Z. X. LI, S. X. ZU, AND M. F. MAO. The Effect of Six Azolla Selections Under Three Management Practices on the Yield of Paddy Rice. In Biological Nitrogen Fixation for Tropical Agriculture, Edited by P. H. Graham, J. Halliday, and P. J. Dart, CIAT, Cali, Colombia.

LUMPKIN, T. A. AND D. L. PLUCKNETT. Azolla as a Green Manure: Use and Management in Crop Production. Westview Press, Boulder, Colorado. 230 pp. 1982.

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NEWTON, J. W. AND D. D. TYLER. Formation and Breakdown of Azolla polypeptides in paddy soils. Proceedings International Workshop on Practical Applications of Azolla for Rice Production, Mayaguez, Puerto Rico, November 17-19, 1982. In press.

NEWTON, J. W. AND D. D. TYLER. Electrophoresis-Radioautography of Polypeptides from Azolla and Symbiotic Dinitrogen-Fixing Algae. Plant Physiol. Suppl. 69 (1982):110.

B. TECHNOLOGIES FOR FOOD AND FEED USES OF FIELD CROPS

1. Rapid Characterization of Yeasts Through Genetic and DNA/DNA Hybridization and Computer Analysis (C. P. Kurtzman)

- a. Specific Objective: Compare hat-spored species of Pichia and Hansenula to determine points of relatedness.

Progress: Species determined similar on standard morphological and physiological tests were compared through DNA relatedness tests. Pichia lindneri and Hansenula minuta exhibited 75% DNA base sequence complementarity, i.e., within the range found between strains of a single species. This finding demonstrates that nitrate assimilation, which was used to separate the two genera, is not of major taxonomic

significance and species in Hansenula H. et P. Sydow 1919 must be transferred to Pichia Hansen 1904.

- b. Specific Objective: Determine hierarchy of relatedness of species within the genus Pichia.

Progress: DNA from the type strains of all known species of Pichia were isolated and purified. Techniques were developed for the isolation and purification of ribosomal RNA in order to determine distant relationships through ribosomal RNA-DNA hybridizations.

Publications:

SLININGER, P. J., R. J. BOTHAST, J. E. VAN CAUWENBERGE AND C. P. KURTZMAN. Conversion of D-Xylose to Ethanol by the Yeast Pachysolen tannophilus. Biotechnol. Bioeng. 24 (1982):371-384.

KURTZMAN, C. P. Fungi--Sources of Food, Fuel, and Biochemicals. Mycologia. Accepted.

KURTZMAN, C. P. Biology and Physiology of the D-Xylose Fermenting Yeast Pachysolen tannophilus. In Advances in Biochemical Engineering, A. Fiechter, ed., Springer-Verlag, Berlin. Accepted.

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KURTZMAN, C. P., R. J. BOTHAST AND J. E. VAN CAUWENBERGE. Conversion of D-Xylose to Ethanol by the Yeast Pachysolen tannophilus. U.S. Patent No. 4,359,534, November 16, 1982.

Other Reports:

KURTZMAN, C. P. Taxonomy and Evolutionary Trends Among the Yeasts. Presented at the U.S. Federation for Culture Collections/American Society for Microbiology Meeting, Atlanta, Georgia, March 7-12, 1982.

KURTZMAN, C. P. Conversion of D-Xylose to Ethanol Under Anaerobic Conditions by the Yeast Pachysolen tannophilus. Presented at American Society for Microbiology Meeting, Atlanta, Georgia, March 7-12, 1982.

KURTZMAN, C. P. Yeast Taxonomy (A Series of Lectures on Conventional and Molecular Classification of Yeasts). Presented at the First

International Course on Taxonomy and Classification of Yeasts, Oeiras, Portugal, September 20-October 15, 1982.

2. Germ Plasm Bank of Microorganisms for Research on Plant Residue Utilization (C. P. Kurtzman)

- a. Specific Objective: Continue operation of the Agricultural Research Service Culture Collection (NRRL) including original and supportive research.

Progress: Microbiology staff members of the Agricultural Research Service Culture Collection (NRRL) continued acquiring, maintaining, and distributing cultures and information; their systematic studies; and their original and supportive research. As of January 1, 1983, the Collection maintained 73,654 strains of molds, yeasts, bacteria, actinomycetes, and algae. During 1982, the Collection distributed 4156 strains, of which 2610 were provided to investigators in the United States and 1546 were sent abroad. Of 259 strains deposited in the patent collection, 48 were from foreign sources; 602 patent strains were distributed to United States researchers, and 393 to foreign. One hundred and fifty strains of bacteria and actinomycetes were identified to species for interested parties.

All strains that were acquired from the "International Streptomycete Project" have now been added to the permanent actinomycete collection. About one-half of the actinomycete strains acquired from Rutgers University (known as the "Waksman Collection") also have been processed and added to the permanent collection. As these strains were incorporated into the collection, a basic characterization for each strain was carried out. Dr. Krassilnikov's translation from the Russian of Ray Fungi--Higher Forms, three volumes, was received and over 30 sets have been distributed to the international public.

- b. Specific Objective: Clarify taxonomy of selected actinomycetes through DNA/DNA relatedness studies.

Progress: A basic method for the isolation and purification of DNA from actinomycetes has been developed. The DNA has now been isolated from all but two of the key strains designated as the type strains of the Pridham 1976 Key. DNA/DNA reassociations studies on these strains have been started. G+C determinations on these strains will be made in the near future.

- c. Specific Objective: G+C determination and DNA reassociation studies of agriculturally significant bacteria will be continued.

Progress: The determination of the G+C contents of DNA from 123 Bacillus circulans strains have been completed. Results showed that the G+C values range from 38 to 60 mol %. Approximately 15%

of the strains had G+C contents of 38-40 mol %, a range in which the type strain fitted. Within this group, 10 of 18 strains showed 90-100% DNA relatedness to the type strain and among themselves. These 10 strains were considered to be representatives of the species B. circulans. Among the remaining strains, 55 could be separated into 16 distinct groups on the basis of high DNA relatedness values. Six groups contained five or six members and the remainder consisted of three or two members. Many strains appeared to be unrelated to any of the other strains examined.

- d. Specific Objective: Initiate DNA reassociation studies of B. globisporus and B. psychrophilus to clarify taxonomic relationship of these two species.

Progress: The DNA relatedness values among four of five B. globisporus strains examined ranged from 90-100%. Likewise, the relatedness values among five B. psychrophilus strains ranged from 90-100%. However, interspecific values were low (7-32%). One B. globisporus strain showed low DNA relatedness (10-30%) with all strains examined. Electrophoretic analyses showed significant differences between the protein components extracted from organisms classified as B. globisporus and B. psychrophilus. These observations strongly suggested that B. globisporus and B. psychrophilus were genetically unrelated and, hence, were distinct species.

- e. Specific Objective: Revision of the description of the species B. circulans.

Progress: Characterization of 123 B. circulans strains have been completed. The results indicated that each of the genetically distinct groups was phenotypically homogeneous; however, the groups also differed from each other phenotypically. The group considered to be representative of the species B. circulans was characterized as gram-variable rods that were motile; produced swollen sporangia; grew anaerobically; were catalase positive; usually grew in 7% NaCl; did not reduce nitrate to nitrite; were oxidase negative; usually hydrolyzed Tween 80; hydrolyzed starch, did not utilize citrate; utilized fumarate and malate; did not produce acetymethylcarbinol; and fermented the common pentoses, hexoses, hexitols, and disaccharides.

- f. Specific Objective: Preparation of a comprehensive compendium of methods for the preservation of microorganisms, cell lines, and viruses will be continued.

Progress: Manuscripts are being edited as received from contributors on this long range project.

Publications:

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KURTZMAN, C. P. Hemiascomycetes. In Synopsis and Classification of Living Organisms, S. P. Parker, ed., pp. 198-201, McGraw-Hill Book Co., New York, 1982.

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NAKAMURA, L. K. Deoxyribonucleic Acid Homologies of Lactobacillus amylophilus and Other Homofermentative Species. Intern. J. Syst. Bacteriol. 32 (1982):43-47.

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Other Reports:

LUSSENHOP, J. AND D. T. WICKLOW. Fungal Decomposer Resource Use and Spore Production: Effects of Competition and Grazing. Presented at Ecological Society of America, American Institute of Biological Sciences, State College, Pennsylvania, August 7-12, 1982.

WICKLOW, D. T., R. KUMAR AND J. E. LLOYD. Germination of Blue Grama Seeds Buried by Dung Beetles. Presented at Ecological Society of America, American Institute of Biological Sciences, State College, Pennsylvania, August 7-12, 1982.

3. Characterization and Classification of Mucorales from Cereal Grains and Their Raw Products (C. W. Hesseltine)

- a. Specific Objective: Collect and preserve isolates of Rhizopus and related genera of the Mucorales from various materials, especially cereal grains and their products.

Progress: Among the numerous isolates of Mucorales added to our germ bank reservoir are representatives of Rhizopus, Amylomyces, and Mucor from Indonesian ragi. Several of the newly isolated Mucor strains are near M. javanicus and will give us a good opportunity to study this poorly known species. We have also isolated several strains of Rhizopus from Teak leaves. The Teak leaves had been lightly sprinkled with tempeh and layered to

preserve traditional inoculum for tempeh making in Java. Other strains newly added to our resources are another representative of the rare species Apophysomyces elegans and five recently described new species of Absidia.

- b. Specific Objective: Classify strains of Rhizopus sp. Study and classify strains of Rhizopus already in the collection to accurately identify those with unusual characteristics and more clearly define species and species relationships. Determine which strains appear intermediate to species as currently distinguished in the R. oryzae-R. arrhizus area and evaluate means to identify them. Initiate a search for a good mating pair for the species R. oryzae.

Progress: Now that hundreds of strains in the R. oryzae-R. arrhizus area have been examined, it is concluded that when using the characteristic most depended upon to separate species, namely sporangiospore size, one can align strains in a near continuum. Strains representing R. microsporus have the smallest and most uniform spore size, strains representing R. delemar have the largest and most varied spore size, while strains representing R. arrhizus and R. oryzae represent intermediate spore size and variation. An initial search for a good mating pair for the species R. oryzae has resulted in no progress; we remain with a pair of strains that erratically form a few scattered zygosporos. New strains, a newly devised technique or both are needed to stimulate greater production of zygosporos for this species. Initial investigations of adapting techniques of extracting DNA from strains of Rhizopus have been highly successful. High molecular weight Rhizopus DNA has been spooled for the first time. This success will lead the way to using DNA reassociation studies to help clarify the taxonomic relationships of species in this difficult genus.

- c. Specific Objective: Ragi studies. Study the microflora of ragi or ragi-like microbial starters from China, Taiwan, Indonesia, and Indochina. Determine the kinds and number of microorganisms present in these starters.

Progress: A large number of representative microorganisms were isolated from ragi and ragi-like preparations. Total counts were as high as 74,500,000 organisms with yeast counts varying from 2,900 to 66,000,000 per gram. Mucoraceous molds showed counts as high as 50,000. Also present in a regular fashion were microaerophilic bacteria with counts to 7,600,000. Representative strains of these three groups of organisms were isolated and considerable characterization done on members of all three groups. The literature on ragi was searched and a review of what is known is partially written.

Publications:

REINHARDT, D. J., I. LICATA, W. KAPLAN, L. AJELLO, F. W. CHANDLER AND J. J. ELLIS. Experimental Cerebral Zygomycosis in Alloxan-Diabetic Rabbits: Variation in Virulence Among Zygomycetes. Sabouradia 19 (1981):245-255.

ELLIS, J. J. AND L. AJELLO. An Unusual Source for Apophysomyces elegans and a Method for Stimulating Sporulation of Saksenaia vasiformis. Mycologia 74 (1982):144-145.

ELLIS, J. J. AND C. W. HESSELTINE. Maintaining Stock Cultures of Rhizopus oligosporus for Tempeh Making. Soyfoods. Accepted.

4. Molecular Genetics Technology for Microbial Production of Plant Polysaccharide-Degrading Enzymes (R. W. Detroy)

- a. Specific Objectives: Increase microbial starch degrading/xylose utilizing enzyme production through employment of cell fusion technology and selective mutational techniques.

Progress: UV mutagenesis of P. tannophilus has produced over 40 mutants that are defective in xylose utilization on agar plates. Testing for enzymatic activity has shown that none of these mutants are defective in the structural genes for xylose reductase or xylitol dehydrogenase.

The hybrid plasmid YEpl3, capable of replicating in both E. coli and S. cerevisiae, was restricted with the endonuclease Bam HI and ligated with BamHI-treated fragments of Pachysolen tannophilus nuclear DNA. The ligated fragments were inserted into host E. coli and the clone bank was screened for identifying markers. The clone bank presently consists of 900 separate individuals.

The transformation efficiency of S. cerevisiae with the hybrid plasmid YEpl3 alone has been increased to a frequency of 1.5×10^{-3} transformants/ug DNA. No transformation has been done using the Pachysolen clone bank. Three relatively stable (75%) fusion products formed during treatment of protoplasts of P. tannophilus and S. cerevisiae with polyethyleneglycol were tested for fermentation rates of glucose and xylose. None exhibited any difference in production from that of wild type Pachysolen.

The yeast, Pachysolen tannophilus, can utilize the pentose D-xylose with accumulation of significant quantities of ethanol. Cell extracts of the organism contain NADPH-linked D-xylose reductase (aldose reductase EC 1.1.1.21) and NAD-dependent D-xylitol dehydrogenase (D-xylulose reductase EC 1.1.1.9). D-Xylose was required for induction of both the D-xylitol dehydrogenase and the D-xylose reductase. Neither enzyme was found in glucose grown cell-free extracts.

An increase in alcohol production by biological fermentation is an important area of current research. Modification of yeasts, by either conventional genetics, cell fusion, or recombinant DNA techniques, offers the possibility of increasing alcohol production. In studies with Saccharomyces cerevisiae, our results show that mitochondrial mutants and the wild type strain have similar growth, alcohol production rates, and total alcohol production when grown under the same conditions. These results indicate that respiratory-deficient mutants of S. cerevisiae do not have a greater capability than the wild type for increased alcohol production.

- b. Specific Objective: Transformation of the cellulose enzyme complex genes into yeast/bacteria for the production of fermentable sugars from cellulose.

Progress: C. lusitaniae and C. wickerhamii were tested for the ability to directly convert cellodextrins (G_1) to ethanol. C. lusitaniae was capable of fermenting only glucose and cellobiose while C. wickerhamii had the unique capacity to efficiently ferment cellodextrins as large cellohexose (G_6). When grown on 54 g/l cellodextrins C. wickerhamii produced 29.2 g/l ethanol in 3-4 days.

Publications:

ALEXANDER, NANCY J. AND R. W. DETROY. Alcohol Production by Mitochondrial Mutants and Wild Type Saccharomyces cerevisiae. Biotechnol. Lett. In press.

FREER, S. N. AND R. W. DETROY. Characterization of Cellobiose Fermentations to Ethanol by Yeasts. Biotechnol. Bioeng. (1982).

FREER, S. N. AND R. W. DETROY. Direct Fermentation of Cellodextrins to Ethanol by Candida wickerhamii and C. lusitaniae. Biotechnol. Lett. 4 (1982):453.

FREER, S. N. AND R. W. DETROY. Direct Fermentation of Chemically Prepared Cellodextrins to Ethanol. Patent pending.

SMILEY, K. L. AND P. L. BOLEN. Demonstration of D-Xylose Reductase and D-Xylitol Dehydrogenase in Pachysolen tannophilus. Biotechnol. Lett. 4 (1982):607-610.

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ALEXANDER, N. J. Genetic Engineering of Yeasts for the Production of Ethanol. Int. Symp. Genetic. Engineering, Sao Paulo, Brazil, December 6-10, 1981.

ALEXANDER, N. J. Application of Molecular Biology Techniques to Bioconversion Microbes. ARS North Central Region Cooperators Conference, Peoria, Illinois, October 17, 1982.

DETROY, R. W. Application of Molecular Biology Techniques to the Efficiency Production of Hydrolytic Enzymes. U. of Zagreb, Zagreb, Yugoslavia, September 12, 1982.

5. Fermentative Utilization of Cane Sugar Bagasses (P.L. 480 Grant - National Research Centre, Cairo, Egypt)

- a. Specific Objective: Study lignocellulosic conversion processes of cane sugar bagasses.

Progress: Under optimized conditions, complete saccharification of the bagasse holocellulose by T. viride 253 enzyme preparation could be achieved by sequential treatment of its hemicellulose and NaOH-treated-cellulose components. Under similar conditions, saccharification of NaOH-treated bagasse was successfully accomplished.

The SCP fermentation studies revealed the formulation of a simple nutritive medium as well as an adequate technique for the cultivation of T. viride 253. Under these conditions, a fodder product of a good nutritional value could be obtained. The amino acid profile of this fodder protein revealed the presence of all essential amino acids. Its content of lysine, threonine, valine and pH-alanine was relatively higher compared with the FAO references. On the other hand, the produced fodder contained lower amounts of methionine and leucine.

C. TECHNOLOGIES FOR FOOD AND FEED
USES FOR ANIMAL PRODUCTS

1. Conversion of Feedlot Wastes Into Feed Supplements by Fermentation with Grain (G. R. Hrubant)

- a. Specific Objective: Continue comparison of microbial profiles of cattle wastes from cattle on diets containing/not containing rumensin.

Progress: Laboratory examination of waste from nonrumensin-fed cattle was completed. Within the groups (1) total aerobes, (2) arthrobacters, and (3) fecal coliforms, there is no apparent correlation between rumensin resistance and resistance to any of the other 10 antibiotics tested. Except for the insensitivity of gram negatives toward rumensin, no pattern of antibiotic resistances emerged. Selective resistance of the lactic acid bacteria to rumensin was marked. Only 12% of the isolates were resistant to 2 mcg rumensin/ml growth medium. Lactics that grow at 45°C, but

not at 15°C, are sensitive to rumensin; those that grow at 15°C, but not at 45°C, are resistant to rumensin. In nonselective media, essentially all lactics are resistant to streptomycin, neomycin, and usually polymyxin B. Lactics, resistant to rumensin, characteristically show increased resistance to streptomycin and resistance to a broader spectrum of the nine other antibiotics tested. Lactic acid bacteria, both gas producers and nonproducers, which are resistant to rumensin, produce alcohol in media containing rumensin but not in media containing streptomycin or any other antibiotic. Cultures producing ethanol in rumensin-containing media continue ethanol production when transferred into a like medium, but cease ethanol production when transferred into a rumensin-free medium.

- b. Specific Objective: Determine the growth pattern of Salmonella cholerae-suis inoculated into silage fermentations of swine waste and corn.

Progress: Salmonella derby was chosen instead of S. cholerae-suis because a late available study on Salmonellae incidence showed the former organism occurred in 37.5% of the isolations from slaughter plants in six states (two-thirds of U.S. production) but no isolations of the latter organism were found. Salmonella derby, inoculated into swine waste combined with corn and packed into laboratory silos, gave initial numbers (per dry g) of 1.2×10^6 . No Salmonellae were found at 8 hr or in 15 other samples taken over a period of 2- to 3-week fermentations. A comparable disappearance of fecal coliform bacteria, a group used to indicate the possible presence of Salmonellae, was also recorded with starting numbers of 4.0×10^6 , reducing to 2.3×10^3 at 1 day and never detected afterwards. The growth pattern of the controlling group of lactobacilli, which caused the rapid elimination of Salmonellae and fecal coliform bacteria, commenced with counts of lactic acid bacteria of 3.7×10^7 . This number increased 40-fold at 8 hr to become, and remain, the dominant group of organisms in the silage fermentation. The lactobacilli rapidly produced organic acids to drop pH almost two units to near 4 pH at 1 day and reached the lowest level of 3.69 at 13 days. Fecal odor, present at inception of the fermentation, became silage-like after 1 day.

Publication:

WEINER, B. A. Silage Fermentation of Swine Waste Combined with Corn. Appl. Microbiol. Biotechnol. 16 (1982):39-44.

2. Feeding Trials of Animal Waste-Grain Fermented Foods Using Recycled Waste (Cooperative Agreement - University of Illinois)

- a. Specific Objective: Compare basic fermentation parameters of pilot-scale fermentor with laboratory results.

Progress: A directed fermentation was started using laboratory-prepared inocula. Four strains with known antibiotic resistance markers were added at 0 time to the pilot-scale fermentor. After a 20-hr batch fermentation, the three chambers were sampled and continuous fermentation begun. Waste from the test animals was also taken. Cultures showed that 79% of the lactics in the three fermentor chambers were like the inocula, while only 4% of the lactics from the waste were similar. A sample of waste taken from the test animals 7 weeks later showed that 23% of the lactics in the waste were like those of the original added inoculum. "Infection" of the cattle gut with the laboratory isolates is occurring.

- b. Specific Objective: Initiate feeding trials.

Progress: The continuous fermentor is built and has been operating successfully for 7 weeks. Each of the three chambers has a capacity of 300 lb; daily output is 200 lb of corn-waste ferment. Finishing cattle--six per group, approximately 900 lb each--are being fed the test diet (ferment) and control diet [90% (85% corn + 15% silage) + 10% soy + vitamin-mineral mix]. After 42 days, the test animals gained 0.5 lb/day more than the control. Test animals averaged a daily weight gain of 2.92 lb on 19.93 lb of feed intake while control animals averaged a daily weight gain of 2.40 lb on 16.52 lb of feed intake. Feed efficiencies were essentially the same (test animals, 6.82 vs. control animals, 6.88), although the test diet used about 12% less feed grain than that of the control diet.

D. BIOMATERIALS SCIENCE

1. Microbiological and Enzymatic Procedures for Conversion of Agricultural Residues and Biomass (R. W. Detroy)

See Northern Agricultural Energy Center, A.2.

2. Innovative Fermentation Technology for Alcohol Production (R. W. Detroy)

See Northern Agricultural Energy Center, A.3.

E. TECHNOLOGIES AND PRODUCTS TO INCREASE EXPORTS OF AGRICULTURAL PRODUCTS

1. Soybean Foods of the Traditional Oriental Type for the Export Markets (H. L. Wang)

- a. Specific Objective: Effect of soybean variety on the yield and quality of tofu.

Progress: Tofu was made on a laboratory scale from 5 U.S. and 5 Japanese soybean varieties grown under the same environmental conditions. Significant variation among varieties was observed for protein and oil content of the beans, size of the beans, amount of water absorbed at complete hydration, protein concentration of soymilk, fresh tofu yield, tofu protein on dry basis, and tofu hardness. Analyses of variance indicated that none of these differences was associated with the origin of the soybean varieties (U.S. vs. Japanese).

Protein and oil contents of the beans directly affect those of the soymilk, which in turn affect the protein and oil contents of the resultant tofu. Since protein and oil contents of the beans are negatively correlated, tofu made from a variety having high protein content would result in tofu having a higher protein to oil ratio than tofu made from a variety with less protein. The yield of tofu, however, is positively correlated with protein recovery during processing, but not with the protein content of the beans. Differences in the hardness of tofu are associated with water content.

Processing conditions in making tofu have previously been found to greatly affect yield and quality. Varietal differences seem to exert minor effects. Since composition and color of tofu are affected by soybean variety, varieties with a light hilum and high protein content would be preferred.

- b. Specific Objective: Investigate the effect of storage on the quality of soybeans.

Progress: This study is completed. After 18 months of storage at ambient temperature, percentage of germination reduced to 70-80%, the weight of cooked beans decreased slightly and the hardness of cooked beans increased. There were no significant changes in protein solubility and trypsin inhibitor activity.

- c. Specific Objective: Investigate the amyloglucosidase production by the molds used in Oriental food fermentation.

Progress: Amylomyces rouxii NRRL 5866 was selected for enzyme study. The mold grown on a solid substrate of a rice and bran mixture (80:20) with 100% H₂O added yielded the highest amount of amylo-glucosidase. Isolation and purification were accomplished by water extraction, ammonium sulfate fractionation, gel filtration, and DEAE-Sephadex chromatography. The last step removed all the pigment and also completely separated amyloglucosidase from α -amylase and proteolytic enzymes.

- d. Specific Objective: Investigate the environmental conditions on the fatty acid composition of Chlorella minutissima.

Progress: Marine microorganisms have been reported to contain relatively high amounts of polyunsaturated fatty acids, especially 20:5 (w-3) and 20:6 (w-3). Fatty acid 20:5 (w-3) has been known as one of the precursors for prostaglandin synthesis. This study was initiated by a visiting scientist from Japan. Effects of light intensity, growth temperature and salt concentration in the medium on the fatty acid composition of C. minutissima were studied. Light intensity affects the growth rate of the organism, but not the cellular fatty acid composition. Growth temperature and salt concentration affect the growth as well as the fatty acid composition. Percentage of 20:5 (w-3) acid was increased in the cells grown at 20°C than 25°C. The supplementation of NaCl also increased the percentage of 20:5 (w-3) acid. Of the total cellular fatty acid of C. minutissima grown in salt-enriched medium, 40% is 20:5 (w-3) acid.

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F. NATURAL TOXICANTS AND MICROBIAL TOXINS

1. Germ Plasm Bank of Microorganisms for Research on Microbial Toxins
(D. T. Wicklow)

- a. Specific Objective: Continue operation of the Agricultural Research Service Culture Collection (NRRL) including original and supportive research.

Progress: Mycology staff members of the Agricultural Research Service Culture Collection (NRRL) continued acquiring, maintaining, and distributing cultures and information; their systematic studies; and their original and supportive research. As of January 1, 1983, the Collection maintained 73,654 strains of molds, yeasts, bacteria, actinomycetes, and algae. During 1982, the Collection distributed 4,156 strains, of which 2,610 were provided to investigators in the United States and 1,546 were sent abroad. Of 259 strains deposited in the patent collection, 48 were from foreign sources; 602 patent strains were distributed to United States researchers, and 393 to foreign. Four hundred and thirty-six strains of molds and yeasts were identified to species for interested parties.

- b. Specific Objective: Develop and evaluate new media to stimulate sporulation of molds to preserve germ plasm by lyophilization. Initiate storage of cultures in refrigerators over liquid N₂.

Further new media will be devised using nutrients, especially from cereal grains and other crop plant parts. Poorly or nonsporulating fungi will be grown on those media to evaluate their sporulation-stimulating potential for use in strain preservation by lyophilization. Nonsporulating and other cultures that do not survive the lyophilization process will be grown out on appropriate media under optimal conditions. Agar plugs from these cultures will be placed aseptically in sterilized containers and stored in the refrigerator over liquid N₂. Material will be processed for both short-term storage and viability testing and for longterm storage.

Progress: Fifty nonsporulating or poorly sporulating strains of fungi were grown on three newly devised media using a rice base. Pycnidial initials were formed by a strain of Ascochyta and conidia were formed by a strain of Helminthosporium. The latter strain was successfully lyophilized for the first time. Three-hundred and forty-eight strains of fungi were grown out under appropriate conditions. Agar plugs permeated with hyphae were aseptically cut out and submerged in cryotubes containing sterilized 10% glycerol or 5% DMSO as a preservative. These preparations were placed in the vapor phase of N₂ in a liquid nitrogen refrigerator for storage. An indexing system was set up for the inventory control system to locate strains when needed and to record storage data. It has already been necessary to retrieve two strains (one a patent strain) from this refrigerator. Both strains grew out well from their respective stored materials and stock agar slant cultures were renewed.

- c. Specific Objective: Mycological studies of seed caching by desert rodent granivores.

Heteromyid rodents have evolved over the past 11,000,000 years in conjunction with a burrow environment suitable for mold growth and the production of mycotoxins. Interdisciplinary work is underway (O. J. Reichman, Department of Biology, Kansas State University) to understand how heteromyids effectively manage their harvested food resources (seeds). Through a series of laboratory and field studies, we plan to investigate the basic caching economy and cache management procedures of the rodents to identify and analyze changes in the fungal populations (seedborne and rodent inoculated), to examine seed caches for suspected mycotoxins, and for changes in the proximate chemical composition, viability, and fat acidity value of individual seed types. The results of this research may provide clues for the improved management of harvested cereal grains in human agroecosystems in ways related to mycotoxin control and the impact of fermentation technology on the nutritional quality of seeds.

Progress: Mycological studies of rodent seed caches collected near Portal, Arizona, in May and September 1982 have revealed: (1) that seeds stored by rodents may experience the same classic fungal successional pattern as has been demonstrated repeatedly for human grain stores; (2) that the fungal communities on rodent-stored seeds are dominated by several of the important toxigenic fungal species isolated from molded grain; (3) that inoculum in the form of mold spores is carried in the furlined cheek pouches of the rodents and is transferred to seeds that are gathered by the rodent and carried back to the burrow for below-ground storage; (4) that the cheek-pouch mold inoculum is dominated by a few mold species which are not necessarily the species recovered from molded rodent-stored seeds.

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WICKLOW, D. T. Predator Defense Strategies of "Seed-Eating" Fungi. Presented at University of Illinois, Urbana, Illinois, October 25, 1982.

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WICKLOW, D. T. Predator Defense Strategies of "Seed-Eating" Fungi. Presented at University of Minnesota, St. Paul, Minnesota, December 2, 1982.

2. Aflatoxin and Other Mycotoxins in Corn and Other Cereal Grains (O. L. Shotwell)

- a. Specific Objective: Study collection and handling of grain samples to prevent mycotoxin formation after collection and during storage before analysis.

Progress: Corn samples (75) were collected in 1981 from four areas of Georgia and separated into six sets of samples to study the effects varying levels of Monoprop (1 acetic acid + 1 vermiculite). One set of samples was dried in Georgia immediately after harvest. The other five sets were shipped without husking to Peoria; one set was husked and dried immediately upon arrival. The other four sets were husked and stored 6 weeks with varying levels of Monoprop (0, 1%, 2%, and 5%). Samples dried in Georgia before shipping had an

average total aflatoxin level of 198 ng/g. Samples shelled and dried immediately after arrival in Peoria had 640 ng/g aflatoxin. Samples shelled and dried after 6 weeks' storage had average aflatoxin levels of 595 ng/g. Samples stored 6 weeks in the presence of 1%, 2%, and 5% Monoprop had average toxin levels of 376, 350, and 342 ng/g. Aflatoxin B_{2a}, the water adduct of aflatoxin B₁ formed in the presence of acid, was not detected in any sample stored in the presence of Monoprop.

- b. Specific Objective: Continue study in cooperation with Dr. W. R. Burg, Department of Environmental Health, University of Cincinnati, on the potential hazard to agricultural workers from inhaling dusts from aflatoxin-contaminated corn.

Progress: Samples of airborne dusts generated from corn were collected at elevators using total samplers and personal samplers with cutoff filters to separate the respirable particles (less than 10 μ) from the nonrespirable particles (10 μ or more). The ratios of aflatoxin levels in respirable particles, nonrespirable particles, and bulk corn from which dust particles were generated were studied. The ratios were not consistent between lots of corn.

- c. Specific Objective: Develop and evaluate new approaches to aflatoxin analysis of corn and mixed feeds.

Progress: Mixed feeds and corn used in animal-feeding trials to obtain FDA approval for the process of detoxification of aflatoxin-contaminated corn by ammoniation were reanalyzed. Samples of Clemson Chicken Rations, Georgia Swine Rations, Rat Chow Rations prepared from animal tissues and corn used in cattle-feeding studies were analyzed by the method developed last year for mixed feed and the CB method for corn. Analytical results were similar to those obtained at Athens, Georgia, during the time of the feeding trials. Analysis of corn samples by the mixed feed method has not been successful because extracts of Midwest corn obtained by the method contain impurities that enhance the fluorescence of aflatoxin on thin-layer chromatography (TLC) plates. High-performance TLC is being applied to the quantitation of aflatoxins.

- d. Specific Objective: Develop and evaluate both screening and quantitative methods of analyzing cereal grains and feeds for mycotoxins, particularly Fusaria toxins.

Progress: An analytical method for the quantitation of DON in wheat was evaluated and modifications made which permitted a significant increase in the number of samples analyzed per day without loss of sensitivity or accuracy. Confirmation of toxin contamination was done by MS/MS. The ultraviolet (UV) spectra of standard DON in methanol and acetonitrile shows a maximum at 224 nm

and a coefficient of extinction of ca. 6500 at 25 µg/ml. HPLC of standard DON with UV detection showed that 10 ng could be detected and detector response was linear from 0.10 to 2.0 µg toxin. These procedures in conjunction with GC of the trimethylsilyl derivative, are used to determine purity of DON preparations.

- e. Specific Objective: DON for analytical standards and biological studies will be prepared from field-inoculated corn or from in vitro fermented substrates.

Progress: DON production by 50 *Fusaria* isolates was determined. Inoculated cracked corn was incubated for 3 weeks (1 week at 25°C and 2 weeks at 15°C) and toxin production determined by GC of the trimethylsilyl derivative of the isolated DON. Toxin production ranged from 1 ppm to 211 ppm with 20 strains producing 10 ppm, 29 strains producing 10 to 50 ppm and 1 strain producing 211 ppm. Substrate and time effects were tested on one high-toxin producer. Production yields decreased in the order rice, corn, wheat, and barley. Also, maximum toxin production occurred within 3 weeks (for rice and corn) and then decreased by 10-50% by the end of 7 weeks. Maximum toxin production occurred at 5 and 7 weeks for wheat and barley, respectively.

- f. Specific Objective: Continue study on the transmission and elimination of aflatoxins in animal and poultry tissues.

Progress: In a cooperative project with J. L. Richard, ARS National Animal Disease Center, Ames, IA, two groups of five steers were fed a ration containing corn naturally contaminated with 800 ng/g aflatoxin--one group for 17.5 weeks and one for 15 weeks. A third group of five steers were fed uncontaminated rations. The feeding trials for all three groups lasted 17.5 weeks. The group fed contaminated rations for 15 weeks was switched to a noncontaminated diet the last 2.5 weeks. The aflatoxin concentration in the rations increased from 350 to 455 ng/g during the course of the study. All steers were killed and tissue and fluid samples were taken for analysis. Aflatoxins B₁ and M₁ were detected during the experimental period in blood and urine of all steers given contaminated diets; however, there were no effects on weight gains, feed efficiency, or on immune phenomena such as lymphoblastogenesis and antibody production. There was a waning of the delayed cutaneous hypersensitivity in the steers given aflatoxin diets. In the animals switched to noncontaminated rations at 15 weeks, aflatoxins B₁ and M₁ disappeared from all tissues and fluids except rumen contents (0.09 ng B₁/g) at the time of necropsy. All tissues taken from the steers that remained on the aflatoxin diet the entire period had B₁ and/or M₁ present (0-0.37 ng B₁/g, 0.11-4.82 ng M₁/g) with the most total aflatoxin in the kidney (5.91 ng/g) and liver (1.44 ng/g). Urine assays provided a monitor of detectable aflatoxin in the tissues.

- g. Specific Objective: Study occurrences of mycotoxins in cereal grains in the field under different climatic conditions and in storage.

Progress: The procedure developed for the determination of DON in wheat was used in a study of 1982 scabby wheat. Hard red winter wheat samples (161) were collected in areas of Kansas and Nebraska in which scabby wheat had been observed. The samples were representative of the wheat harvested in the selected areas. Correlations between grading factors and toxin levels were studied; the grading factors with the highest correlations were total damaged kernels, total defects, and scab-damaged kernels, including shrunken and broken kernels. The identity of DON in 17 samples selected randomly was confirmed by MS/MS.

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- STUBBLEFIELD, R. D. Distribution of Aflatoxins in Tissues from Cows Ingesting Artificially Contaminated Feed. Presented at Association of Official Analytical Chemists Meeting, Washington, D.C., October 25-28, 1982.
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3. Metabolites of Toxin-Producing Fungi Found in Corn and Other Cereal Grains (M. D. Grove)

- a. Specific Objective: Determine the end products formed by ammoniation of the aflatoxin model ketocoumarin on corn.

Progress: Experiments utilizing the triple-stage quadrupole mass spectrometer or MS/MS were carried out to search for small amounts of ketocoumarin degradation products in a corn matrix. A partially refined extract prepared from an ammoniated ketocoumarin on corn germ reaction mixture was examined by MS/MS for three compounds: a ketophenol, a diketone, and dimethoxyphenol. Mass chromatographic plots of the intensities of three major daughter ions arising from the protonated molecular ions allowed the ketophenol and diketone to be identified. The ketophenol is analogous to aflatoxin D₁. Finding the diketone was unanticipated because of its instability in ammonia to produce dimethoxyphenol which has previously been shown to undergo oxidative ammoniation to give several products. The absence of dimethoxyphenol confirms earlier results obtained using GC/MS.

- b. Specific Objective: Determine reactivity of the dihydrofuran portion of aflatoxin under conditions used for ammoniation of corn.

Progress: Larger quantities of chemical intermediates essential for synthesis of the model test compound were prepared. The final elimination reaction step of the synthetic pathway does not produce the desired 2,3-unsaturated bisfuran when the ethyl carbonate ester is used as a precursor. A direct dehydration of the 2-hydroxy derivative by P₂O₅ also failed to give the desired compound. The acetate ester did produce small amounts of product, according to GC/MS data. More acetate ester is being produced for use in preparing the 2,3-unsaturated product.

- c. Specific Objective: Study Fusarium strains for their ability to produce metabolites that cause animal feeds to be unpalatable.

Progress: A substance produced by F. moniliforme NRRL 6322 and refused by mice when added to their drinking water was estimated to be present in laboratory cultures in amounts equivalent to 40 mg/kg vomitoxin as determined by a comparison of water consumption. The refusal factor of NRRL 6322 has been consistently produced on a corn grit medium. A several-fold concentration of this refusal factor has been achieved by passage through charcoal columns and by sequential phasing from polar to less polar solvents.

- d. Specific Objective: Assess toxicoses of farm animals thought to be caused by consumption of moldy grains or feeds made from infected grains.

Progress: Several samples of feed and corn, which were associated with swine syndromes of refusal to eat, vomiting, poor weight gains in feeder pigs, and gestation problems in sows were investigated for trichothecenes. Eight samples were found to contain 2 to 10 µg/g of vomitoxin. Four samples of poultry feed which were involved in either feed refusal or beak lesions were examined for 12 trichothecenes, particularly T-2 toxin, but none were found.

- e. Specific Objective: Develop methods for detoxification of cereals that contain refusal factors.

Progress: Heating an aqueous solution of T-2 tetraol at 121°C and 15 psi results in formation of several products. The two major compounds have been identified as isomeric hydrates resulting from attack by water at the 9-double bond of the epoxytrichothecene nucleus followed by epoxide ring opening to give a hexaol. T-2 toxin is resistant to hydrate formation under these conditions, presumably due to hindrance presented by the bulky 3-methylbutyryl moiety at position-8. Preliminary testing of the hydrate in the mouse drinking water bioassay indicates it is readily accepted in contrast to the potent refusal produced by T-2 toxin and T-2 tetraol. An attempt to effect a microbial transformation of vomitoxin by lactobacilli failed to degrade the vomitoxin as shown by GC/MS analysis.

- f. Specific Objective: Determine the cause of equine leucoencephalomalacia.

Progress: In a study done in cooperation with Dr. G. Long, Purdue University, 23 mice received a diet consisting of 50% ground rodent chow and 50% *F. moniliforme* corn culture. The female mice began to die after 4 days and the males after 9 days. The dead mice were emaciated, but had no gross lesions. The most likely cause of death was reduced feed intake. Twenty mice were fed a diet of 80% rodent chow and 20% *F. moniliforme* corn culture. The mice were sacrificed after 60 days. The only tissue in which significant differences were noted was the liver. Hepatocellular nuclei from treated animals were about twice the size of controls. A sample of corn implicated in the death of several horses in Georgia from leucoencephalomalacia was examined. An extract was obtained which was toxic to mice upon intraperitoneal injection. No moniliformin, T-2 toxin, vomitoxin, or diacetoxyscirpenol could be detected.

- g. Specific Objective: Develop methods for the production of metabolites of *Fusaria* to facilitate their availability for use in toxicological evaluations.

Progress: Two vomitoxin-producing isolates of *Fusaria* were grown on cracked corn for 1 to 8 weeks at 15, 20, 25, 28, and 32°C.

Maximal production was calculated to occur at 40 days and 30°C for F. graminearum NRRL 5883 and at 41 days and 26°C for F. roseum NRRL 6101. These optima were determined from response surface graphs related to temperature and time. Utilizing high-performance liquid chromatography as the final fractionation step, 133 µg of 95% pure vomitoxin was isolated per gram of corn fermented with F. graminearum. A radial pressure liquid chromatographic method for assessing the purity of vomitoxin was developed. A 1.5-g sample of vomitoxin was furnished by Dr. G. Long, Purdue University, for experiments involving swine. Two grams each of vomitoxin and T-2 toxin were supplied by Dr. R. J. Cole, Dawson, Georgia, for studies in poultry.

- h. Specific Objective: Identify secondary metabolites produced by Fusaria.

Progress: An antibiotic(s) substance from a Fusarium sp. A-15,483 that inhibits some molds as well as gram-positive bacteria has been produced in quantities sufficient for limited biological and chemical studies. The antibiotic also inhibits seed germination of grass and cucumber. Mass spectrometry indicates the main component to have a composition of $C_{23}H_{46}O_6$.

- i. Specific Objective: To study distressed corn for mycotoxins.

Progress: Three samples of distressed (moldy) corn to be used in alcohol fermentation were evaluated for mycotoxins. One sample was found to contain diacetoxyscirpenol and vomitoxin. A second contains a refusal factor as shown by the mouse drinking water bioassay. The third contains substances lethal to mice by intraperitoneal injection. No trichothecenes were detected in the latter two samples.

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4. Origin and Ecology of Mycotoxin-Producing Fungi in Grain (D. T. Wicklow)

- a. Specific Objective: Compare the distribution of aflatoxins in conidia versus sclerotia of A. flavus and A. parasiticus.

Progress: Total aflatoxin levels in conidia and sclerotia varied considerably both within (intrafungal) and among strains. A. flavus NRRL 6554 accumulated the highest levels of aflatoxin (conidia: B₁ 84,000 ppb; G₁ 566,000 ppb; sclerotia: B₁ 135,000 ppb; G₁ 968,000 ppb). Substantial aflatoxin levels in conidia could place at risk those agricultural workers exposed to dust containing large numbers of A. flavus conidia. Cellular ratios of aflatoxin B₁ to aflatoxin G₁ were nearly identical in conidia and sclerotia even though levels of total aflatoxins in these propagule types may have differed greatly. Aflatoxin G₁ was detected in sclerotia of all A. flavus strains but in the conidia of only one strain. Each

of the A. parasiticus strains examined accumulated aflatoxin G₁ in both sclerotia and conidia. Current evolutionary theory predicts an increase in the chemical defense systems of fungal sclerotia, propagules critical to fungal survival.

- b. Specific Objective: Determine mechanism of sclerotium germination in A. flavus and A. parasiticus.

Progress: Sclerotium germination in A. flavus and A. parasiticus is sporogenic with conidial apparati (yellow-green conidial heads) produced directly from exposed sclerotium surfaces. Five of seven sclerotium-producing strains chosen for this study formed yellow-green conidial heads on individual surface-sterilized sclerotia within 48-72 hr incubation (25-37°C) on saturated sand. Germination is also reported for sclerotia that were incubated on nonsterile field soil. In one experiment, buried sclerotia (1 cm) migrated to the soil surface as a result of intermittent watering during which soils become inundated. These newly exposed sclerotia also germinated. Because A. flavus is sensitive to soil mycostasis, sporogenic germination represents an important mechanism enabling dissemination of inoculum. Eventual control of preharvest A. flavus infection in crops/regions where aflatoxin is a problem may require agronomic practices designed to reduce the importance of sclerotia as a source of primary inoculum.

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5. Detection and Quantitation of Mycotoxins and Natural Toxicants by Mass Spectrometry (M. D. Grove)

See Horticultural and Special Crops Laboratory, F.2.

6. Microbial Species Interactions and Development of Aflatoxin in Preharvest Corn (Cooperative Agreement - University of Wisconsin)

- a. Specific Objective: Biotron studies included experiments designed to measure the effects of fungal species interactions on susceptibility of kernels to A. flavus invasion and subsequent aflatoxin contamination.

Progress: A. flavus is capable of infecting undamaged corn kernels and producing aflatoxin. Silk-inoculated ears of four commercial dent cultivars (upper midwest Corn Belt = Funks G-4141A, DeKalb XL-12; southeastern coastal plain = Pioneer 3369A, DeKalb 72B) showed substantial variation in kernel infection (%) but no aflatoxin contamination when growth room temperatures corresponded to typical upper midwest conditions (photo period, 14 hr; temperature, $30 \pm 1^\circ\text{C}$ day/ $20 \pm 1^\circ\text{C}$ night; humidity, $82 \pm 3\%$). At these same temperatures, however, aflatoxin was produced in individual wound-inoculated kernels. Undamaged kernels located adjacent to the wound-inoculated kernels had uniformly high levels of aflatoxin irrespective of wounding date (e.g., 7, 14, or 21 days after silk). It is argued that wounded kernel tissue provides A. flavus with a resource base from which it can increase its inoculum potential and thus its ability to infect adjacent intact kernels.

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7. Application of The Trickle Ammonia Process to Drying Corn at Ambient Temperatures (Cooperative Agreement - University of Minnesota)

- a. Specific Objective: Determine optimum conditions for on-farm and elevator use of trickle ammonia process for drying corn at ambient temperatures to conserve energy.

Progress: The movement of ammonia through a 3000-bushel bin of corn was followed with cartridges containing adsorbant that changed from white to purple when exposed to ammonia. Studies on the microflora indicated that ammonia was effective in controlling fungi. Bacteria and yeast levels were reduced. The most resistant fungi were F. moniliforme and Alternaria. The corn was dried from 25.4-13.7% moisture.

- b. Specific Objective: Determine effect of trickle ammonia process on feed values of corn.

Progress: Studies on feeding the corn dried by the trickle ammonia process to dairy cattle indicated there was no adverse effect on feed intake or milk production. The beef cattle feeding trial is almost completed; the results have not been analyzed.

- c. Specific Objective: Demonstrate advantages of the trickle ammonia process to farmers and elevator owners.

Progress: The process has been described to farmers and elevator owners on 20 occasions where grain storage was discussed. There have been four presentations to farmers, elevator owners, and scientists exclusively on the trickle ammonia process. One farmer, as a result of a presentation to a feed cooperative organization, is drying corn containing 30% moisture using the process.

Report:

MERONUCK, R. A. Made 24 presentations to farmers and elevator owners in Minnesota.

8. Mycotoxins in Food and Feedstuffs (P. L. 480 Grant - Pakistan Council of Scientific and Industrial Research Laboratories, Lahore)

- a. Specific Objective: Continue screening foods and feeds in Pakistan for mycotoxins.

Progress: Aflatoxin contamination studies of feedstuffs which showed positive results were continued. Work on bright greenish-yellow fluorescence as a presumptive indicator of aflatoxin in artificial decontamination of feeds is in progress.

HORTICULTURAL AND SPECIAL CROPS LABORATORY

E. B. Bagley, Chief

Research Leaders: R. Kleiman, J. A. Rothfus, and C. R. Smith, Jr.

A. BREEDING AND PRODUCTION FOR FORAGE CROPS FOR HAY, PASTURES AND OTHER USES INCLUDING TURF

1. Chemicals in Tall Fescue Affecting Livestock Health and Forage Utilization (S. G. Yates)

- a. Specific Objective: Prepare acids or lactones that occur in toxic fescue hay that are suspected toxicants and test in calves.

Progress: Gram quantities of 2,4-dihydroxybutyrolactone have been prepared. This is a major component of the toxic anion fraction of fescue hay.

- b. Specific Objective: Explore the feasibility of using tandem mass spectral analysis as a tool in comparing toxic to nontoxic forages and hays.

Progress: Twelve known alkaloids were obtained and, by means of tandem MS-MS, 10 were identified within a crude matrix consisting of a barley extract. Quantitation in a fescue extract is needed before using the technique.

Publications:

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B. INTRODUCTION, CLASSIFICATION, MAINTENANCE, EVALUATION
AND DOCUMENTATION OF PLANT GERMPLASM

1. Isolation and Identification of Allelochemicals from Uncultivated Plants (R. Kleiman)

- a. Specific Objective: Screen seed extracts for plant-to plant allelopathic activity.

Progress: A germination bioassay for allelopathic compounds using velvet leaf seed was developed. With leads suggested by a complete review of the literature on plant-plant allelopathy, seed from over 100 species from our wild seed collection were tested for germination inhibition. Of these, 53 showed some inhibitory activity. Two potent inhibitory species, Lycopus europaeus and Carica papaya, were identified. Extracts from seed of these species were fractionated and the fraction further tested. Benzylisothiocyanate isolated from Papaya showed great activity. An active crystalline compound from L. europaeus is currently being characterized.

- b. Specific Objective: Characterize seed oils for useful and/or novel compounds.

Progress: Preliminary experiments indicate that alkoxy-acyl combination in complex wax ester mixture can be solved by tandem mass spectrometry (MS/MS). Unsaturated species can be quantitated by deuterium reduction to saturated compounds. The method is applicable to microgram quantities. Forty new species have been identified as containing gamma-linolenic acid. Gamma-linolenic has been proposed as a prostaglandin precursor.

- c. Specific Objective: Analyze potential new crops species and new cultivars of existing crops in cooperation with plant breeders.

Progress: Twenty-six samples of Cuphea, collected in the wild were analyzed for their fatty acid composition. These analyses added to the base of knowledge on Cuphea germplasm. In a continuing effort to develop rapeseed as a crop for the Northwest United States, samples were analyzed in cooperation with Washington State and Oregon State Universities for oil content, fatty acid composition, and glucosinolate content. Over 100 crambe samples were analyzed for oil content and fatty acid composition. These samples were provided by T. Austin Campbell, ARS Agricultural Research Center, Beltsville, MD.

- d. Specific Objective: Develop process of forming superior coatings from epoxy oils. Evaluate additional catalysts and polyfunctional monomers in coatings formulations with Vernonia oil. Test oil with doubled oxirane content as a film former. Evaluate thermal analysis as an analytical tool in film forming tests.

Progress: Evaluation of physical-chemical properties of Vernonia oil was initiated by differential scanning calorimetry (DSC). At low temperatures, liquid-to-solid (exotherm) and solid-to-liquid (endotherm) transitions were observed at 227° and 284°K, respectively. No changes in these transitions were observed as a result of heat treatments between ambient and 600°K (10°K/min scans from 300°K to final temperatures of 400, 450, 500, 550, 600°K). However, the transitions disappeared (exotherm) or changed markedly (endotherm) when the thermal scan was carried to 650°K, the apparent change in oil character (polymerization, decomposition) occurring above 625°K. These thermal treatments bracket the region (423 to 473°K) where films or coatings have been successfully prepared on steel panels using neat or pigmented (TiO₂) Vernonia oil (bake times of 10-60 min).

- e. Specific Objective: Assist in the commercialization of crambe in Kentucky-Tennessee-Missouri region.

Progress: Maintenance, renewal, and increase of crambe seed stocks are assured for another year through a cooperative agreement signed in August with Murray State University in Murray, KY, which also provides for modest research on salient production problems. Thirty acres of crambe will be produced in 1983 for seed purposes. Insufficient quality seed in 1982 caused private interests to attempt production of high-erucic rapeseed in geographic extremes of the continental U.S.

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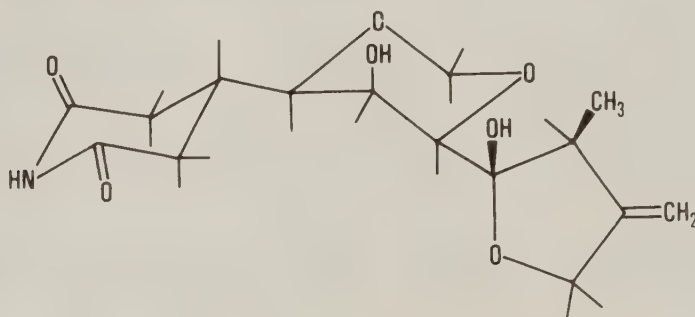
2. Biologically Active Plant Constituents for Pest Control and Medicine (C. R. Smith, Jr.)

- a. Specific Objective: Detect biological activity in extracts of seed and other plant parts through screening, both in-house and by outside cooperators.

Progress: During the past year, 48 new extracts have been prepared and submitted to a National Cancer Institute contractor. Most of these were submitted during the last 2 months of the year so that bioassay results are not yet available.

- b. Specific Objective: Isolate and characterize active constituents with confirmed biological activities.

Progress: From the highly bioactive extract of Sesbania drummondii seed have been isolated two compounds responsible for its antitumor activity. One of these, sesbanimide, has been fully characterized by a combination of chemical reactions, NMR spectra, and X-ray crystallography, and has the following structure:



The other active compound, isosesbanimide, appears to be epimeric with sesbanimide at C-10 or an adjacent chiral center. Both hydrogenation and acetylation of sesbanimide have proven to be complex processes, each yielding several identifiable products. Those isolable in sufficient quantity are being bioassayed.

Fractionation of the ethanol extraction of Diploclisia glaucescens (Menispermaceae) is at an advanced stage. Solvent partitioning followed by HPLC procedures have yielded ten fractions which are being bioassayed with European corn borer larvae. The main insecticidal component has been identified as β -ecdysone, a highly oxygenated steroid. At least four related compounds are present, one of which is makisterone A.

Fractionation of the ethanol extract from 20 kg of Diarrhron vesiculosum (Thymeleaceae) is at an advanced stage. Solvent partitioning, open column chromatography, and HPLC techniques have yielded an antileukemic substance which appears to be one of the daphnane series of diterpenes.

- c. Specific Objective: Identify constituents of oats that attract the saw-toothed grain beetle (Oryzaephilus surinamensis L.).

Progress: The carbonyl fraction of oat volatiles has been further fractionated by preparative gas chromatography, and various isolated compounds have been bioassayed for attractancy in a pitfall chamber. The most potent attractants were (E)-2-nonenal and (E,E)-2,4-nonadienal. On the other hand, several saturated aldehydes (e.g., hexanal, octanal) were attractive, but only at 100-1000 times greater concentrations than the two unsaturated aldehydes named above.

- d. Specific Objective: Identify the resistance factors of pest-resistant apples.

Progress: Our cooperator, Dr. H. Goonewardene (ARS, Purdue University) found previously that certain ethanol extracts of his Golden Delicious apples significantly deterred feeding of apple maggots. These extracts have since been fractionated by solvent partitioning, preparative TLC, and gas chromatography. There are certain compositional differences between corresponding fractions from resistant and non-resistant varieties. Work is now underway to determine the nature of the differences.

- e. Specific Objective: Determine chemical basis for difference between varieties of corn which differ in their field resistance to the rice weevil (Sitophilus oryzae).

Progress: Fractionation of extracts examined earlier by Dr. Luis Gomez (Dr. J. G. Rodriguez' laboratory, University of Kentucky) has been carried out by solvent extraction, TLC, and GLC; resulting fractions have been bioassayed by Dr. Gomez' procedure. Results are suggestive of location of bioactive material, but are not satisfactorily consistent. More work must be done to clarify the situation.

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3. Major Fatty Acids from Indian Seed Oils and Their Possible Industrial Use (P. L. 480 Grant - Aligarh Muslim University, Aligarh, India)

- a. Specific Objective: Screening Indian flora for unusual and potentially useful lipid constituents; synthesis of new and potentially useful derivatives.

Progress: Among the classes of fatty acid derivatives synthesized during the past year are: γ -lactones, iodine nitrate and nitrosyl chloride addition products, and phosphorus-containing esters. Thermal degradations of isoricinoleic acid have been studied. Seed oils of the following plants are repellent to the red flour beetle (Tribolium castaneum): Polyalthia longifolia, Annona squamosa, Heliotropium supinum, and Semecarpus anacardium. In all cases, the repellency residues in the unsaponifiable portion of the oil.

Publications:

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C. PHYSIOLOGICAL AND BIOCHEMICAL TECHNOLOGY TO IMPROVE CROP PRODUCTION

1. Plant Cell and Tissue Culture for the Bioproduction of Valuable Chemicals (N. E. Delfel)

- a. Specific Objective: Investigate the regeneration of whole plants from cultured cells.

Progress: Carrot (Daucus carota) cultures were induced to form embryoids and to germinate in vitro. Such structures were found to consist of meristematic cells surrounded by so-called callus cells, as described by others. Similar structures were induced in Cephalotaxus harringtonia and Chenopodium quinoa callus cultures by lowering the auxin level while raising that of the cytokinin. The precise ratio of the two was more important than the absolute level of either.

- b. Specific Objective: Study the specificity of alkaloid membrane-transport by cells in suspension culture.

Progress: Adequate control cultures of Datura were developed without difficulty, but growth characteristics of Cephalotaxus cells in suspension culture precluded meaningful comparison studies on alkaloid transport. Effort was transferred to investigations of secondary metabolite production in Chenopodium.

- c. Specific Objective: Develop screening technique for assaying the saponin production of cloned cells.

Progress: Quinoa seeds and pieces from callus cultures were incubated on blood agar plates for one or two days and the size of the resulting halo noted. In general, results were inconclusive: The halo formed with seeds was small while that surrounding the callus was not correlated with the size of the callus piece. Attempts to further refine this approach or to develop alternate

screening methods were postponed until a suitable, sensitive analytical method was developed with which to correlate results.

- d. Specific Objective: Develop an analytical technique for pentacyclic triterpenes.

Progress: A GLC procedure based on an OV-101 column and temperature programming was capable of resolving the following compounds either reported to be, or likely to be present in Chenopodium spp.: β -amyrin, oleanolic acid, hederogenin, and echinocystic acid. Erythrodilol had the same retention time as oleanolic acid. All five triterpenes were separated by TLC using $\text{CHCl}_3/\text{MeOH}$ and detected using the Liebermann-Burchard test for sterols.

Reports:

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2. Control of Plant Morphogenesis and Energy Transfer System Structure and Action in Crop Plants (J. A. Rothfus)

- a. Specific Objective: Correlate leaf pigment ratios with crop plant development and performance.

Progress: In cooperative work with M. Duysen, North Dakota State University, Fargo, ND, analyses to identify appropriate methodology and chloroplast fractions prefaced developmental studies on wheat seedlings. Pigment-protein complexes from chloroplast thylakoids of seedling leaves were separated by two different gel electrophoretic procedures and analyzed by a previously developed HPLC method with particular attention to quantitating levels of neoxanthin, violaxanthin, lutein, and carotene. Carotene was a principal pigment constituent of electrophoretic bands associated with photosynthetic reaction center complexes, but it occurred at much lower levels in light-harvesting complexes and may well be absent from such complexes in vivo. Neoxanthin and violaxanthin were principal carotenoids in light-harvesting complexes. Lutein, which was present at significant levels in all complexes also predominated in light-harvesting complexes. Studies correlating corn genotype, development, and performance with morphology and pigmentation of photosynthetic apparatus were extended to include additional plant accessions and detailed chemical and microscopic measurements on isolated mesophyll and bundle sheath cells augmenting leaf disk pigmentation and microstructure studies conducted previously. Analyses of collected data are underway.

- b. Specific Objective: Study photomorphogenesis in soybeans.

Progress: Efforts to detect real protein differences in soybeans during germination after exposure to selected wavelength light produced extremely variable results, perhaps due to difficulties in controlling non-light elements of the growth environment. Comparison of pigment-protein complexes from leaf chloroplasts of different soybean genotypes, however, pointed out fundamental differences in the functional morphology of green versus yellow and senescent soybeans. Analyses of complexes from 8- and 10-week plants showed that chlorophyll deficiency correlates with low levels of light-harvesting complexes and that the ratio photosystem I/photosystem II is a function of genotype ranging from ca. 2.5 in the normal green genotype to ca. 1.1 in the most severely deficient varieties. Levels of photosystem II are higher in the deficient plants. Likewise, chloroplasts from senescent leaves show higher levels of photosystem II when compared to normal mature chloroplasts from green leaves on the same plant. Senescence thus apparently favors the initial destruction of light-harvesting complexes followed by deterioration of photosystem I in remaining reaction center complexes. These results agree with other types of photosystem I/photosystem II determinations but correlate negatively with conclusions that photosystem II is primarily located in appressed regions of grana. It is clear from electron micrographs of chloroplasts from these normal and chlorophyll-deficient varieties that the chlorophyll-deficient genotypes contain considerably less grana stacking and more stroma thylakoids than the normal.

- c. Specific Objective: Investigate light-triggered phosphorylation of membrane constituents associated with photosystem II.

Progress: Thylakoid membrane protein phosphorylation affects photochemical reactions of photosystem II. Incubation of thylakoids in the light with adenosine triphosphate leads to: (1) an increase in the amplitude of three components (4-6 μ s, 25-45 μ s, and 280-300 μ s) of delayed light emission after a single flash without any change in their kinetics, (2) a reduction of the flash-dependent binary oscillations of chlorophyll a fluorescence yield associated with electron transfer from the primary quinone acceptor, Q, to the secondary quinone acceptor, B, (3) an increase in the B/B ratio resulting from an increase in stability of the semiquinone anion during dark adaptation, (4) no change in the redox state of the plastoquinone pool as determined by flash-induced photooxidation of the photosystem I reaction center, P₇₀₀. All of these effects, which are reversible upon dephosphorylation of the thylakoid membranes, can be explained by a protein phosphorylation-induced stabilization of the bound semiquinone anion, B⁻. Such increased stability could logically be due to an alteration in the accessibility of an endogenous reductant to B. The exact reason for semiquinone anion stabilization remains uncertain, but this work clearly identifies an additional controlling link in the chain of reactions associated with the regulation of photosynthesis.

- d. Specific Objective: Continue work on the effects of bicarbonate on photosystem II reactions.

Progress: Additional insight into the role of bicarbonate in regulating photosynthesis was sought by examining analogies to another carbon dioxide responsive biosystem; namely, carbonic anhydrase. Accordingly, known inhibitors of carbonic anhydrase were tested for effects on photosystem II activity in chloroplasts. Formate inhibition of photosystem II turnover rates increases as the pH of the reaction medium is lowered. Bicarbonate ions inhibit photosystem II turnover rates. Acetazolamide inhibition of photosystem II increases as light intensity decreases, indicating a lowering of quantum yields in the presence of the inhibitor. Imidazole inhibition of photosystem II increases with pH in a manner suggesting that the unprotonated form of the compound is inhibitory. Formate, bicarbonate, acetazolamide, and imidazole all inhibit DCMU-insensitive, silicomolybdate-supported oxygen evolution, indicating that the site(s) of action of the inhibitors is at, or before, the primary stable photosystem II electron acceptor, Q. This inhibitory effect of bicarbonate would appear to operate antagonistically to the enhancing effect of bicarbonate on quinone-mediated electron flow that was observed in previous work. The presence of bicarbonate could thus allow efficient electron flow away from the photosystem II reaction center and simultaneously decrease the turnover rate of the oxygen evolution mechanism. Bicarbonate depletion would inhibit electron flow but enhance the turnover rate of the oxygen-evolving mechanism. Such an antagonistic couple may play an important role in photosystem II regulation. The results suggest that the photosystem II complex normally does not have bicarbonate bound to it and that it functions optimally when the anion binding site is vacant. By analogy to carbonic anhydrase, it is easy to speculate that inhibitory bicarbonate binds to photosystem II at manganous ions, perhaps displacing water molecules that would otherwise act ultimately as substrate for the oxygen-evolving mechanism.

- e. Specific Objective: Examine effective absorption cross sections of photosystems I and II in corn.

Progress: Mesophyll and bundle sheath chloroplasts were isolated successfully from known corn genotypes. Photosystem stoichiometry measurements on these different chloroplasts, using instrumentation constructed previously, showed that agranal bundle sheath chloroplasts are deficient in photosystem II reaction centers relative to the granal mesophyll chloroplasts. The accuracy of these measurements is critically dependent on the extinction coefficient for Q, the primary electron acceptor of photosystem II. To obviate question of data based on a generally accepted value for Q, an alternate method was developed (collaboration with A. Melis, University of California, Berkeley) to determine the extinction

coefficient of Q by comparing the theoretical rate of electron flow at photosystem II, based on the concentration and kinetics of photosystem II, with measured rate of electron flow to natural and artificial electron acceptors. The extinction coefficient for Q measured in this manner was not significantly different from the accepted value.

- f. Specific Objective: Characterize pigmented elements of the light-harvesting pigment-protein complex of Phaeodactylum tricornutum.

Progress: Yellow and green particles isolated from the LHPP complex of P. tricornutum were characterized further in terms of size, pigment content, and amino acid composition. The yellow particles, which are hexameric, contain ornithine while the green particles, which are tetrameric, do not. Pigment contents also differ. Relative molar proportions of chlorophyll a, chlorophyll c, fucoxanthin, and total other accessory pigments are 3:1:6:2 in the yellow particles and 5:1:3:1 in the green. Variation in centrifugal sedimentation behavior of the yellow particles during their isolation suggests that the hexameric aggregate may actually exist in vivo as extended discoid structures like those typical of phycobilisomes. Consistent with this conjecture, HPLC data show the possible presence of dodecamers related to the yellow particles. The LHPP structure favored by these studies is thus a macromolecular array perhaps larger than 1 million daltons, which consists of at least two types of particles: green ones of 45 K daltons containing 4 polypeptide chains with 8 pigment molecules and yellow ones of 70 K daltons containing 6 polypeptide chains with 12 pigment molecules. Amino acid analyses further indicate the presence of at least two different kinds of polypeptide subunits in the yellow particles.

- g. Specific Objective: Complete studies on energy transfer in model systems.

Progress: The energy transfer system in Phaeodactylum tricornutum was modeled by adsorbing pigments from the algae on a reversed-phase support. At a spacing of 30 square nanometers per chlorophyll and 22 square nanometers per fucoxanthin, a synthetic mixture of fucoxanthin-chlorophyll a and the P. tricornutum extract both gave only the emission spectrum of chlorophyll a (major emission at 682 nm) when excited at any wavelength ca. 400 to 500 nm. An excitation contribution at 465 nm indicated energy transfer in both synthetic mixture and algal extract. Comparing relative excitation intensities at 465 nm showed that, in this model system, not all the energy transfer in the algal pigments could be accounted for by fucoxanthin. Spectra from wider pigment spacings showed that the energy transferring entity in the extract has excitation and emission peaks at 465 and 650 nm, respectively.

- h. Specific Objective: Reexamine photosystem stoichiometry in soybean mutants.

Progress: Previous work with chloroplasts from a chlorophyll-deficient mutant soybean demonstrated that the relative photosynthetic unit size was smaller in both photosystems II and I compared to photosystems in normal green soybean chloroplasts, and that the photosystem II/photosystem I ratio was lower in the yellow mutant than in the normal soybean. In collaboration with A. Melis, University of California, Berkeley, photosystem stoichiometry in the soybean mutant was remeasured, and Q reduction kinetics and P₇₀₀ oxidation kinetics were also determined. As before, absorption cross sections were smaller for both photosystems II and I in the yellow mutant than in the green soybean, but photosystem II/photosystem I ratios were greater in the chlorophyll-deficient soybean than in the normal soybean.

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D. TECHNOLOGIES FOR FOOD AND FEED USES FOR FIELD CROPS

1. Composition and Properties of Seed Lipids for Foods and Feeds (J. A. Rothfus)

- a. Specific Objective: Characterize thermal properties of polyunsaturated acids and related esters and triglycerides.

Progress: Characterization of additional acids, esters, and partially oxygenated triglycerides completes qualitative thermal data on 136 selected compounds representing different combinations of structural elements encountered in seed lipids. Thermodynamic measurements and, in some instances, kinetic data also have been accumulated on most phase transitions exhibited by these materials. This collection of data provides a context within which the quantitative analysis of certain lipid thermal properties in terms of structural elements seems quite possible. Endergonic contributions due to aliphatic interactions are distinguishable from those due to hydrogen bonding and geometrical isomerism. Likewise, fusion entropies that are higher for methyl esters than for the corresponding acids (even though the esters melt at lower temperatures) may be accounted for quantitatively by hydrogen bonding through methoxyl groups. Changes in thermal properties with increasing chainlength in odd and even chainlength saturated triglycerides are attributable to shifts from one controlling structural feature to another. And, in the same context, the substantially different thermal behavior of cis and trans monoenoic acid triglycerides is yielding to similar analysis. On average, fusion enthalpies observed for cis triglycerides are lower than those for trans triglycerides by an amount consistent with conformational restrictions imposed by the geometric differences. Thermodynamic and kinetic effects on thermal behavior are especially evident with 5-cis-methyl eicosenoate, which exhibits two different solid to liquid phase transitions (267 and 275°K) depending how fast and how far the molten ester is cooled. Furthermore, this ester appears to follow different polymorphic paths into and out of low temperature phases if cooling exceeds critical transition temperatures. These studies emphasize the importance of quantitative and kinetic data in extending such analyses of structural contributions in homogeneous systems to analyses of intermolecular interactions in heterogeneous systems.

- b. Specific Objective: Investigate polymorph stabilization in known single acid triglycerides.

Progress: Procedures developed for the preparation of intermediate (β') forms of tristearin proved equally useful in further investigation of β' form stability in odd chainlength triglycerides. Raman and infrared spectroscopy, X-ray diffraction, and differential scanning calorimetry studies established that trimargarin (C_{17}), like tristearin (C_{18}), can exist in four solid-state phases that are each stable at room temperature. Furthermore, corresponding phases of the odd and even chainlength triglycerides are identical in terms of Raman vibrational band frequencies and intensity ratios. Aliphatic end group interactions may affect the stability of these

phases because Raman spectral changes that correlate with phase transitions occur at wavelengths attributable to methyl group vibrations. Apparently, however, lateral packing between aliphatic chains, which appears the same in odd and even triglycerides, does not account for the tendency of odd chainlength triglycerides to melt from a β' form rather than a β form, as would be typical of even triglycerides. Conformation or packing variations at other loci should thus also be considered. X-ray low angle scatter data confirm the fundamental odd versus even disparity. Long spacings for trimargarin do not coincide with values interpolated from those for even-membered triglycerides, and angles of hydrocarbon chain tilt calculated from the long spacing data indicate less variation between β' and β phases of the C_{17} triglyceride than those of tristearin: 90, 66.5, 65.0, and 63.7° for α , β'_2 , β'_1 , and β respectively for C_{17} ; 90, 72.2, 59.2, and 50.4 for the respective C_{18} phases.

c. Specific Objective: Continue computer modeling of triglycerides.

Progress: Molecular interaction energies computed previously for 72 different combinations (nine subcell arrangements of molecules involving eight different conformations) of even chainlength and odd chainlength β' form, "bent," triglycerides were reexamined in greater detail for evidence of differences from the α form, "tuning fork" structure, which allows several equally preferred subcell arrangement combinations and apparently does not discriminate between even or odd chainlengths. Total molecular interaction energies were computed by summing intermolecular atomic interactions for β' molecules in positions of extreme (β -like) or lesser angles of tilt with respect to end group planes. For triarachidin, C_{20} triglyceride, only 13 lesser tilt forms had acceptable levels of repulsive interactions and eight of these could be excluded because of unfavorable asymmetric molecular spacing. For trinonadecanoin, C_{19} , only two lesser tilt forms had acceptable interactions and symmetry. In a population of C_{20} molecules, multiple intermediate (β') forms that differ spacially but not energetically might facilitate the rapid transition to β forms, which is typical of even chainlength triglycerides but not odd triglycerides. If such a subtle selective mechanism exists, preliminary evidence suggests that its operation most likely depends upon events prior to or during β' phase formation. Additional modeling experiments indicate, thus far, that with β' molecules bent in the opposite direction by a different chain 2 conformation, the energetic differences between even and odd chainlength structures are not so obvious. Effort was therefore initiated to augment the intermolecular space relationships modeling with approaches based on observable thermodynamic characteristics of phase excitation and transition.

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2. Soybean Analysis for Improved Quality (R. Kleiman)

- a. Specific Objective: Determine oil and protein content of soybean samples in order to develop improved varieties.

Progress: About 15,000 samples were received from public soybean breeders throughout the United States and Canada. These samples were examined for their oil and protein content by the infrared reflectance method, and the data were reported to the cooperating breeders.

- b. Specific Objective: Provide fatty acid composition of selected soybean samples in order to lower the linolenic acid content through plant breeding.

Progress: Fatty acid composition was determined on 6,700 soybean samples. Included in these gas chromatographic analyses were 2,000 germplasm collection samples from the northern collection. These analyses complete the fatty acid profile of the northern collection. Analysis of 4,700 chemical mutation samples from J. Wilcox, ARS, Purdue University, yielded several low linolenic acid (18:3) samples. These were progeny from mutants found low in 18:3 last year. Six germplasm lines found to be 5% or below in 18:3 were regrown in six different locations, two in Illinois, Georgia, Iowa, Indiana, and Maryland. Only one, PI 361088B, maintained its low 18:3 level. Five bushels of this line was grown for us by an Illinois farmer. It had an 18:3 content of 4.2%.

- c. Specific Objective: Develop rapid analytical procedures for soy protein in order to raise the methionine and cystine content through breeding.

Progress: A gas chromatographic procedure using cyanogen bromide indicates adequate results in the rapid analysis for cysteine. Further testing is in progress to compare the results from this method with the standard method.

- d. Specific Objective: Continue support of NRRC projects by providing amino acid analyses.

Progress: About 130 samples were analyzed for amino acids. Many of the samples required special techniques.

- e. Specific Objective: Study control mechanism which regulates lipid biosynthesis in plants, and begin purification of plant acyl carrier protein.

Progress: Acyl carrier protein has been purified from spinach using salt fractionation, ion exchange chromatography, and HPLC. The protein has been crosslinked using glutaraldehyde to increase its immunogenicity. Facilities, equipment, and protocols for antibody production using rabbits have been established.

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3. Basic Studies of Physical Properties of Soy and Cereal Based Food Ingredients (E. B. Bagley)
 - a. Specific Objective: To examine response of doughs to large non-linear strains in the ERD testing mode and determine whether behavior in the non-linear response region provides a more sensitive method for characterizing component interactions than does the response in the linear region.

Progress: Evaluation of dispersions of starch granules heated in water within the gelatinization range (e.g., 60-75°C for wheat starch) has continued to provide the basic information needed for an understanding of the physical behavior of a variety of food materials including doughs. The dispersions have been studied both in water-limited and non-water limited cases. When water is not limiting, the viscosity behavior was examined by a rotational instrument at the temperatures 60°C and room temperature. It was discovered that even though the granules are not disrupted or solubilized in these dispersions, yield points occur when the dispersions are cooled below 35°C. The yield points have been studied as functions of concentration, temperature and sample history. Dispersions in the water-limited region, not accessible to the usual viscometric studies, could be characterized by deforming the dispersions in shear. These dispersions showed essentially solid-like behavior and stress-strain diagrams, carried out to the fracture point unexpectedly showed a yielding phenomenon similar to that encountered in some metallic and polymeric solids. In addition, it was discovered that large normal stresses are generated in shear (stresses perpendicular to the direction of deformation). This provides very fundamental, new information on

these systems which in principle will allow complete and fundamental characterization of material properties which is vital both to food quality factors and to evaluation of the effect of processing on food properties and provides guidance towards an improved experimental approach to the evaluation of the properties of food materials.

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4. Fundamental Studies on Separation of Starch, Protein, and Lipid of Corn (E. B. Bagley)

See Biomaterials Conversion Laboratory, B.1.

E. UTILIZE, MANAGE, AND CONSERVE SOIL FERTILITY FOR INCREASED PRODUCTION AND NUTRITIONAL QUALITY OF PLANTS AND ANIMALS

1. Improve and Implement the Determination of Isotopic Nitrogen in Soil Samples (R. Kleiman)

- a. Specific Objective: Cooperate with ARS soil scientists in determining amount of ^{15}N in samples developed through nitrogen X tillage experiments.

Progress: Over 3000 samples from five different groups in Nebraska, Illinois, and Minnesota, were analyzed for atom % ^{15}N . These results were obtained through the use of an automated N_2 generation system.

- b. Specific Objective: Improve automatic procedure for conversion of NH_4Cl to N_2 and introduction into the mass spectrometer for analysis of atom %N.

Progress: The automated N₂ generation system was greatly improved in both number of samples per day which could be analyzed and in the reliability of the data obtained. By modification of a laboratory fraction collector, sample capability was increased from 70 per day to 200 per day. Many other modifications, both in software and hardware, resulted in increased reliability and speed of operations, allowing a sample to be run every seven minutes completely under computer control. Results are stored on tape and outputted to a teletype, a copy of which is sent to the cooperating scientists.

F. NATURAL TOXICANTS AND MICROBIAL TOXINS

1. Natural Toxicants in Horticultural Crops and Cruciferous Feeds (M. E. Daxenbichler)

- a. Specific Objective: Evaluate biological activity of selected toxicants that occur in cruciferous and umbelliferous vegetables in significant amounts.

Progress: Preparation of gram quantities of 1-cyano-2-hydroxy-3,4-epithiobutanes to carry out a 2-year study in rats is in progress. These isomers are demonstrated liver and kidney toxicants, but low-level, chronic effects are not known. Four nitriles derived from crucifer vegetables are being evaluated as to the in vitro kinetics of biotransformations mediated by rat liver microsomes: 1-cyano-4-methyl-sulfonylbutane, 1-cyano-3,4-epithiobutane, 1-cyano-2-hydroxy-3-butene, and 1-cyano-3-methylsulfinyl-propane.

- b. Specific Objective: Provide convenient sources of natural toxicants needed for future testing.

Progress: A seed increase of Eruca sativa (45 lb) has been obtained. Arabis turrita and Isatis aucheri var. vellifera did not set seed satisfactorily. These seeds provide a convenient and concentrated source of glucosinolates found at lower levels in vegetables, and will be used in animal feeding trials.

- c. Specific Objective: Provide data base of vegetable glucosinolate content for evaluation of newly developed cultivars and to expand germplasm useful in plant breeding.

Progress: A survey of Oriental vegetables of Brassica juncea and B. campestris has been completed. A survey of Oriental Raphanus sp. has also been completed. This broadens the germplasm information base available to plant breeders.

- d. Specific Objective: Survey levels of faltarinol, faltarindiol, and myristicin in market carrots. Explore the toxicants in parsnips and water celery (Oenanthe javanica).

Progress: The survey of market carrots for year-to-year variation of four commercial cultivars in several locations has been completed. One cultivar each of parsnips and of water celery have also been analyzed for these three toxicants. Myristicin is present at levels of less than 1 ppm in most accessions and would not appear to be a health problem. Levels of other suspected toxicants are high enough to be of possible concern, and feeding trials are projected.

- e. Specific Objective: Gain information on storage and processing effects on the presence of these toxicants in carrots.

Progress: Carrots stored at 4°C for 1 year lost only 10% of both falcarinol and falcarindiol. Two samples of commercially canned carrots contained 6 ppm falcarinol, 26 ppm falcarindiol and no myristicin. Storage thus appears to have minimal effect on the levels of these components, but further work is needed on the effects of processing on these toxicants.

- f. Specific Objective: Study mechanism of formation of toxic w-epithio-alkyl cyanides from glucosinolates.

Progress: Methodology has been devised for a novel chemical analysis of the epithio moiety and for assay of the myrosulfatase enzyme. These techniques are being used in the mechanism study not yet completed.

Publications:

DAXENBICHLER, M. E., W. P. SCHROEDER, and G. F. SPENCER. (+)-5-Allyloxazolidine-2-thione, an enantiomer of Turnip Antithyroid Factor Isolated from Berteroa incana (L.) DC. J. Agric. Food Chem. 30(1982):1248-1250.

NISHIE, K. and M. E. DAXENBICHLER. Hepatic Effects of R-Goitrin in Sprague-Dawley Rats. Food Chem. Toxicol. 20(1982):279-287.

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YATES, S. G. and R. E. ENGLAND. Isolation and Analysis of Carrot Constituents: Myristicin, Falcarinol, and Falcarindiol. J. Agric. Food Chem. 30(2)(1982):317-320.

Other Reports:

PETROSKI, R. J. A Titrimetric Assay for Glucosinolate Sulfohydrolase. American Society of Pharmacognosy Meeting, Pittsburgh, Pennsylvania, August 1-5, 1982.

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2. Detection and Quantitation of Mycotoxins and Natural Toxicants by Mass Spectrometry (R. D. Plattner)

- a. Specific Objective: Implement mass spectroscopy/mass spectroscopy (MS/MS) methodology program to analyze toxins.

Progress: The new tandem mass spectrometer was installed and is operational. Experiments with 12 ergot peptide alkaloids demonstrated that all isomers could be differentiated by MS/MS analysis of collisionally activated dissociation (CAD) daughters. The applicability of the method was tested by analyzing an ergot-contaminated feed sample that had caused problems in swine. Ten of the peptide alkaloids were positively identified as present in the sample. The levels of several of them were sufficiently low that they had been overlooked by conventional wet chemistry methods. MS/MS analysis was applied to the two Fusarium mycotoxins, deoxynivalenol (DON) and zearalenone. Both could be detected in a simple solvent extract of ground grain samples at 1 ppm concentration levels underivatized and without any sample cleanup. Detection in the low ppb range for DON required sample cleanup using a procedure similar to that used for electron capture gas chromatography (GC) analysis. The method was used to confirm results of electron capture GC analysis for DON in a survey of part of the 1982 wheat crop. Various Fusarium mycotoxin and aflatoxin standards were studied by MS/MS. Both positive and negative ion chemical ionizations were studied and the CAD daughters of both parents and fragments were studied at pressures from 0.1 to 3 mtorr and various collision energies. Aflatoxin B₁ could be detected from simple solvent extracts of contaminated grain as low as 10 ppb, but substantial matrix effects indicate quantitative work will require some sample cleanup. Sample cleanup, using procedures that are suitable for high pressure liquid chromatography (HPLC) analysis, improves detection limits to the sub-ppb range and data indicates MS/MS could be a nice complementary analysis procedure to HPLC for determination of trace levels of aflatoxins and metabolites in tissue samples. Two products from ammoniation of an aflatoxin model ketocoumarin on corn were detected in a partially refined extract by MS/MS.

- b. Specific Objective: Analyze toxins by gas chromatography/mass spectroscopy (GC/MS).

Progress: Detection limits for determination of Fusarium mycotoxins zearalenone, zearalenol, DON, and T-2 toxin by GC/MS of the trimethylsilyl derivatives in the electron impact and chemical ionization modes were determined. Confirmatory data for quantitative analysis of these mycotoxins in various matrices were provided. GC/MS analyses aided in structure determination and identification of unusual glucosinolate aglucons from Cruciferae. Quantitative data from GC analyses were confirmed by GC/MS for the toxicants myristicin and falcarinol in Umbellifers.

Reports:

PAYNE-WAHL, K. L., R. D. PLATTNER, G. A. BENNETT, and R. E. ENGLAND. Determination of Fusaria Mycotoxins in Grain Matrices by Mass Spectrometry. Midwest Regional Meeting of the Association of Official Analytical Chemists, Ames, Iowa, June 2-3, 1982.

PLATTNER, R. D., G. A. BENNETT, and J. R. B. SLAYBACK. Detection of Fusarium Mycotoxins in a Grain Matrix by Quadrupole MS/MS. American Society for Mass Spectrometry, Honolulu, Hawaii, June 6-11, 1982.

PLATTNER, R. D., G. A. BENNETT, R. D. STUBBLEFIELD, and J. R. B. SLAYBACK. Identification of Aflatoxins by Quadrupole MS/MS. American Society for Mass Spectrometry, Honolulu, Hawaii, June 6-11, 1982.

3. Inheritance of Glucosinolates in Crucifer Vegetables (Cooperative Agreement - University of Wisconsin)

- a. Specific Objective: Gain information on the inheritance of specific glucosinolates found in cruciferous vegetables.

Progress: Individual plants of Brassica campestris exceptionally high (>776 ppm as glucose) or low (<171 ppm) in total glucosinolates are ready for use as parental clones. Development and characterizations of rapid-cycling crucifer stocks continue; genomes include Aaa·1, Bbb·1, ABaabb·1, and Rrr·1. Hybrids containing male-sterile B. juncea cytoplasm have been prepared for breeding experiments.

Report:

HILL, C. B., P. H. WILLIAMS, D. G. CARLSON, and H. L. TOOKEY. Genetics of Glucosinolates in Crucifers. Crucifer Newsletter #7, EUCARPIA Volunteer Group. 1982.

4. Biological Effects of Potential Toxicants from Glucosinolates (Cooperative Agreement - Colorado State University)

- a. Specific Objective: Gain information on the sub-acute toxicity and accompanying cellular lesions in laboratory animals given glucosinolate-derived aglucon products of significance in cruciferous vegetables.

Progress: Livers of rats fed 150 ppm of 2S-1-cyano-2-hydroxy-3,4-epithiobutanes showed early changes in biliary epithelial hyperplasia with karyomegaly and cytomegaly. By 90 days, the liver lesions include well-developed biliary proliferation and portal fibrosis, and kidney lesions of the proximal tubules were evident. Mice are considerably more resistant to the toxicant than are rats. This work was of particular interest and significance to toxicant research because quantitative aspects of the cellular response were evaluated using computer-assisted microscopic measurements of cell size.

Report:

GOULD, D. H. and M. E. DAXENBICHLER. Liver and Kidney Alterations Induced by Toxic Nitriles from Cruciferous Plants. American College of Veterinary Pathologists Meeting, Atlanta, Georgia, November 2, 1982.

OILSEED CROPS LABORATORY

T. L. Mounts, Chief

Research Leaders: E. A. Emken, E. N. Frankel, J. P. Friedrich
E. H. Pryde, and W. J. Wolf

A. TECHNOLOGIES FOR FOOD AND FEED USES FOR FIELD CROPS

1. Effects of Vegetable and Animal Trypsin Inhibitors in Long-Term Animal Feeding Studies (Cooperative Agreement - University of Minnesota)

- a. Specific Objective: Immunochemical characterization of tumor antigens associated with pancreatic hyperplasia.

Progress: Since one of the main objectives of the University of Minnesota study is to ascertain whether early biochemical markers might serve as indicators of subsequent formation of nodules and adenomas, some pertinent biochemical measurements were made during the feeding stage prior to the formation of pancreatic nodules. Size and secretory activity of the rat pancreas, as measured by trypsin and chymotrypsin activity was significantly correlated with trypsin inhibitor activity (TIA) in the diet. However, use of a non-invasive peptide test, that measures trypsin and chymotrypsin activity in the intestinal tract, revealed that enzymatic pancreatic secretion was greatly reduced in rats exhibiting high tumorigenic incidence as compared to animals fed high levels of TIA prior to the appearance of nodules. The percent recovery of para-aminobenzoic acid (PABA) in the urine rising from the intestinal hydrolysis of the peptide ranged from 43-49% in rats fed the high TI diets from 7 to 48 weeks and then decreased to 24% as the incidence of nodules increased during continued feeding. This decrease in PABA secretion during hyperplastic nodule formation is indicative of impairment in the secretory function of the pancreatic acinar cells in spite of a continued TI-induced signal for hypersecretion.

Since there is a highly significant correlation between the average number and size of nodules in the pancreas and TIA, these results suggest that TI-induced hyperplastic nodules lead to neoplastic changes. Work is in progress to isolate antigens specifically associated with the histopathological changes occurring during ingestion of raw soy flour. In vitro culturing of the tumor cells derived from rats fed raw soybeans with high TIA has been successful. A high antigen titer is expected to facilitate its isolation and characterization. Work is in progress to propagate the tumor cells in vitro and verify in vivo tumorigenicity of these cells by injection into controlled rats.

- b. Specific Objective: Initiate a chronic feeding study with mice and hamsters.

Progress: The transition from TI-stimulated pancreatic hypersecretion to cellular hyperplasia and neoplasia represents a great unknown. The ease of such transitions may vary among animal species and humans. For several reasons, the mouse and golden Syrian hamster were selected as alternative species for study at the University of Minnesota. At the ARS Western Regional Research Center, histopathological examination of pancreatic tissue of mice and hamsters sacrificed after 9 to 12 months of feeding is underway. In the interim, sizes of pancreas of mice fed raw soy flour, heated soy, and casein for 10 months were 1.27, 0.65 and 0.59 g/100 g body weight, respectively. As with mice, the hamster also exhibited a greater sensitivity to the effects of TI in terms of pancreatic function than that observed in rats. These results suggest that the incidence of neoplastic changes should also be much greater for these two species than for the rat.

- c. Specific Objective: Preliminary investigation of the toxicological response of rats fed potato trypsin inhibitor concentrate.

Progress: At the University of Minnesota, a standard 28-day evaluation study has been completed. Results demonstrate that at the same level of TIA in the diet, the potato TI is just as effective as raw soy flour in inhibiting growth, stimulating pancreatic enzyme secretion and causing hypertrophy in weanling rats.

Publications:

HASDAI, A. AND I. E. LIENER. Growth, Digestibility, and Enzymatic Activities in the Pancreas and Intestines of Hamsters Fed Raw and Heated Soy Flour. Nutr. Repts. Int. In press.

Other Reports:

Progress Report, "Biochemical Effects of Vegetable and Animal Trypsin Inhibitors in Long-Term Animal Feeding Studies."

2. Improving Food Quality of Soy Oil Products and Their Stability to Heat-and Light-Catalyzed Oxidation (E. N. Frankel)

- a. Specific Objective: Prepare and characterize secondary oxidation products from polyunsaturated fats and esters oxidized with singlet and triplet oxygen.

Progress: Studies of photosensitized-oxidized methyl linolenate after silicic acid chromatography and polar phase high pressure liquid chromatography (HPLC) identified more secondary oxidation

products. Composition data were completed for systems containing 8.2 to 29.0% mono-hydroperoxides: keto and epoxy dienes (0.4-1%), hydroperoxy epidioxides (0.8-4.9%), hydroperoxy bicyclic endoperoxides (0.1-0.3%), dihydroperoxides (1.0-5.6%), and hydroperoxy bis-epidioxides (0.7-1.6%). Secondary products characterized spectrally included 9-hydroperoxy-10,12,13,15-bis epidioxy-trans-16- and 16-hydroperoxy-10,12,13,15-bis epidioxy-trans-8-octadecenoates, 10,12-, 13,15-, 10,16-dihydroperoxides, 9- and 16-hydroperoxy bicyclic endoperoxides, 9-, 10-, 12-, 13-, 15-, 16-ketodienes and 9,10-, 12,13-, 15,16-epoxy dienes. Unique 10- and 15-hydroperoxides identified in sensitized photooxidized methyl linolenate were shown to undergo further cyclization to produce hydroperoxy bis-epidioxides that may be separated by HPLC into four diastereoisomeric pairs of enantiomers. The 9- and 16-hydroperoxides accumulated as major initial products of oxidation because they lack a cis double bond homo-allylic to the hydroperoxide group, a requirement for 1,3-cyclization and serial cyclization. Dihydroperoxides may be derived from monohydroperoxides by singlet or free radical oxidation.

To prepare 6-membered hydroperoxy cyclic peroxides, photosensitized oxidation of methyl linoleate hydroperoxides was conducted and the oxidation mixture fractionated by silicic acid chromatography with selected fractions separated by HPLC. Products characterized spectrally included 9-hydroperoxy-10,13-epidioxy-cis-11- and 13-hydroperoxy-9,12-epidioxy-cis-10-octadecenoates, 8,13- and 9,14-dihydroperoxy octadecadienoates and epoxy compounds. The six-membered hydroperoxy cyclic peroxides are apparently due to Diels Alder addition of singlet oxygen to 9- and 13-hydroperoxides after isomerization of the conjugated systems to the trans,trans configuration. Dihydroperoxides may be derived from the 9- and 13-hydroperoxides by free radical oxidation.

- b. Specific Objective: Develop methodology for study of volatile flavor products from thermal decomposition of secondary oxidation and other oxygenated compounds from polyunsaturated fatty esters.

Progress: Capillary GC-MS methodology was developed to investigate the volatiles produced by thermal decomposition of secondary oxidation materials from autoxidized and photosensitized oxidized methyl linoleate and linolenate. By using a synthetic saturated hydroperoxy cyclic peroxide as a model, the thermal cleavage reactions were elucidated. Main cleavage occurred between the peroxide ring and the carbon-bearing hydroperoxide group. Hydroperoxy cyclic peroxides and dihydroperoxides produced volatiles that were generally similar to those from corresponding mono-hydroperoxides. New volatiles identified included methyl 8-(2-furyl)-octanoate, methyl ketones, and conjugated diunsaturated aldehyde esters. The volatiles identified from the hydroperoxy bicyclic endoperoxide included methyl 13-oxo-9,11-tridecadienoate

and 2,4-heptadienal. Volatiles from six-membered hydroperoxy-cyclic peroxides showed that the most important cleavage is between the hydroperoxy bearing carbon and the peroxide ring. The next most important cleavage is between the ring alkene and ring oxygen bearing carbons. The third most important cleavage is between the ring and ester or alkyl groups. The general fragmentation observed between the peroxide ring and the hydroperoxide-bearing carbon is sufficiently predictable that it can be used as a tool for the structural characterization of these types of cyclic compounds. This work confirms our previous results in providing direct evidence that secondary oxidation products are important precursors of volatile materials that may affect the flavor of foods containing polyunsaturated lipids.

- c. Specific Objective: Modify synthetic cyclic monomers by selective reactions and prepare new cyclic derivatives that can be found in heated fats. Analyze commercial heated fats for cyclic monomers.

Progress: A large-scale synthesis of methyl 9-(6-propyl-3-cyclohexenyl)-8-nonenoate (MPCN) was achieved, providing 37 g (99+% purity by GLC) of this diunsaturated cyclic monomer for further research and biological testing. A sample of MPCN, submitted to the ARS Western Regional Research Center for biological testing (Ames Test), was reported to be non-mutagenic; however, the report noted that the solubility of MPCN was poor at highest concentrations. For future investigations of biological effects and characterization of heated fats, MPCN was either selectively hydrogenated or partially reduced to prepare two positional, monounsaturated cyclic isomers. Various catalyst and reduction systems were evaluated. The new monounsaturated isomers were separated, identified, and analyzed by argentation HPLC and TLC, reductive ozonolysis-gas chromatography, GC-MS, and spectrophotometric methods. A microozonolysis-gas chromatography procedure was developed, which proved that the double bonds in the monounsaturated products were not isomerized by the hydrogenation or reduction methods employed. Each monounsaturated positional isomer was resolved by capillary GLC into its cis- and trans-ring isomers. By determining an ECL value, each of these four isomers was characterized for future identification in heated fat mixtures.

Heated fat samples were obtained from fast food establishments either after known usage or before disposal in cooperation with Professor L. M. Smith, University of California, Davis, CA, under a memorandum of understanding. Gas chromatographic analyses for cyclic monomers and polar/non-eluted thermal oxidation materials varied widely with types and length of use. These commercial samples ranged from 0.1 to 0.6% cyclic monomers, and from 1 to 8% polar/non-eluted materials.

- d. Specific Objective: Study effect of nonglyceride components of crude soybean oil on the photooxidation of polyunsaturated fatty esters.

Progress: Soybean oil unsaponifiables have been further separated by silicic acid chromatography followed by HPLC. Some of the pigments were identified spectrally by comparison with reference chlorophylls and carotenoids freshly prepared from spinach. In addition to known chlorophylls and carotenoids other minor components have been isolated but not yet identified. A quantitative TLC procedure was developed using chromatographic rods (Iatroscan). Oxidation products from photooxidized methyl linoleate were satisfactorily separated and analyzed quantitatively. The effect of sensitizers (chlorophylls) and singlet oxygen quenchers (carotenoids) and other unsaponifiables can now be investigated on a microscale by this quantitative TLC method.

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NEFF, W. E., E. N. FRANKEL, AND D. WEISLEDER. Photosensitized Oxidation of Methyl Linolenate. Secondary Products. *Lipids* 17 (1982):780-790.

Other Reports:

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FRANKEL, E. N. Analytical Approaches to Fatty Acid Autoxidation. Presented seminar at Union-Camp Corporation, Princeton, New Jersey, March 12, 1982.

FRANKEL, E. N. Volatile Lipid Oxidation Products. Presented at Symposium on the Chemistry of Flavor Stability, 73rd Annual Meeting of the American Oil Chemists' Society, Toronto, Canada, May 2-6, 1982.

FRANKEL, E. N. Lipid Peroxidation and Its Biological Consequences. Presented at Symposium on Biochemical Aspects of the Aging Process, 16th Annual Great Lakes Regional Meeting, American Chemical Society, Normal, Illinois, June 7-9, 1982.

FRANKEL, E. N. Chairman. Agricultural and Food Chemistry General Paper Session. 16th Annual Great Lakes Regional Meeting, American Chemical Society, Normal, Illinois, June 7-9, 1982.

FRANKEL, E. N. Biological and Flavor Implications of Lipid Oxidation. Presented seminar at the Western Regional Research Center, ARS, Albany, California, September 21, 1982.

FRANKEL, E. N. Secondary Lipid Oxidation Products and Their Biological Consequences. Presented at 12th Annual Meeting of the American Aging Association, San Francisco, California, September 23-25, 1982.

NEFF, W. E., E. N. FRANKEL, AND D. WEISLEDER. High Pressure Liquid Chromatography of Photosensitized Oxidized Methyl Linolenate. Secondary Products. Presented at 16th Great Lakes ACS Regional Meeting, Normal, IL, June 1982.

3. Nutritional Quality, Safety and Flavor Aspects of Soybean Protein Products (J. J. Rackis)

- a. Specific Objective: Establish role of peroxidase-catalyzed oxidation of nicotinamide adenine dinucleotide (NADH) on the cooxidation of polyunsaturated fatty acid (PUFA) and subsequent generation of off-flavors.

Progress: To prove that PUFA can be cooxidized via peroxidase-catalyzed oxidation of NADH with either Fe^{+2} or Fe^{+3} as cofactor was not feasible since the iron ions not only acted as cofactors for the NADH oxidation, but also catalyzed oxidation of PUFA. On the other hand, either Mn^{+2} or Mn^{+2} -EDTA complex proved to be a cofactor for NADH but did not oxidize PUFA. Therefore, with manganese as cofactor, we can demonstrate that PUFA is enzymically cooxidized via the NADH-peroxidase system. Action of peroxidases on NADH not only generates superoxide anion radical but also hydrogen peroxide. Superoxide dismutase will dismutate superoxide to oxygen and hydrogen peroxide. Superoxide dismutase and catalase added to model systems containing PUFA, NADH, Mn^{+2} , and peroxidase,

either individually or in combination, will halt PUFA cooxidation. Cooxidation of PUFA apparently involves both hydrogen peroxide and superoxide anion radical. With linoleic acid added to the model system, the major reaction product is monohydroxyoctadecenoic acid.

- b. Specific Objective: Identify oxidation products of di 18:3 phosphatidylcholine (PC).

Progress: Unlabeled di 18:2 and di 18:3 PC's were synthesized and rigorously purified by normal and reverse phase HPLC. Multilamellar liposomes were formed and allowed to autoxidize in aqueous solutions. Unexpectedly there was no increase in 234 nm absorbance even after lengthy periods of incubation, yet extensive volatile compound formations could be detected. Accumulation of 234 nm absorbing material occurred after initiation of oxidation by addition of exogenous hydroperoxide fatty acid indicating that at very low levels of peroxide, the β -scission reaction rate is at least as rapid as the rate of oxidation.

- c. Specific Objective: Determine feasibility of mass spectrometry of intact phospholipids.

Progress: Electron ionization (EI) and chemical ionization (CI) mass spectra were obtained on intact phosphatidylcholine (PC) and we found that ammonia CI spectra of PC introduced by direct insertion probe contained molecular ions and ions which defined the fatty acid composition. Analysis of pure molecular species of PC, species containing deuterated methyl groups on the choline, and ^{15}N , ammonia CI allowed identification of the majority of ions found in the mass spectra. A new direct exposure probe was purchased and mass spectra obtained on phosphatidylcholine, lyso phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, phosphatidylserine, phosphatidylglycerol, and N,N dimethylphosphatidylethanolamine. Preliminary results with the new probe indicate that absolute quantitation of molecular species in mixtures is possible when suitable internal standards are used.

- d. Specific Objective: Preparation of 7S and 11S globulins from soybeans and their interaction with phytic acid and iron.

Progress: Crude 7S and 11S proteins were isolated from defatted soybean meal by extraction with 0.03 M tris HCl buffer, pH 7.6, and stepwise precipitation at pH 6.4 and 4.8. Yields were about 16% crude 7S and 5% crude 11S based on weight of starting meal. Ultracentrifugation indicated about 60% purity for the 7S and 80% purity for the 11S fractions. Pure 11S was obtained by chromatography of the crude 11S on Sepharose 6B with 0.1 and 0.5 ionic strength phosphate-NaCl buffers at pH 7.6. Some purification of 7S protein was also achieved. Purification of the proteins was

monitored by gel electrophoresis in the presence and absence of sodium dodecylsulfate.

Samples of 11S and 7S proteins were analyzed for phytate content by an ion-exchange HPLC procedure. Almost no phytic acid was found in the crude 11S fraction while the crude 7S fraction contained 0.3 to 0.9% phytic acid after dialysis against distilled water. These values compare with 1.6% phytic acid found in the starting soybean meal and in a nondialyzed sodium proteinate and with 1.3% found in an isoelectric protein isolate washed three times with distilled water. Phosphorus analysis indicated little phosphorus and therefore little phytic acid in the whey after dialysis and also little difference in phosphorus content of pH 8.0 soluble and insoluble fractions of an isolate after dialysis. Samples of crude 7S and of soybean isolate were submitted to Dr. M. Marini, Northwestern University, (see A.6.) for calorimetric studies of phytate and mineral binding to soy proteins.

- e. Specific Objective: Complete protocols for research on the biochemical efforts of soy protein isolates on long-term rat feeding studies.

Progress: Protocols, designated NRRC-01 and WRRRC Pan #6, to comply with Good Laboratory Practices, have been written and approved. An interim sacrifice of 5 rats fed the high-TI raw soy protein isolate diet for 1 year (August) was made. Histopathological examinations are in progress.

- f. Specific Objective: Development of a laboratory scale process to prepare a soybean trypsin inhibitor (TI) concentrate.

Progress: A trypsin inhibitor concentrate was prepared from defatted soybean meal by water extraction at pH 7.5 followed by stepwise precipitation of 11S and 7S proteins from the extract at pH 6.4 and 5.0, respectively, in the presence of 0.1 N NaCl. The yield of TI concentrate fraction (supernatant pH 5) was about 7% by weight of the starting meal. Trypsin inhibitor activity (TIA) of this fraction was 32 mg/g of starting meal or 440 mg/g of TI concentrate. This represented 87% of the TIA found in soybean meal and 97% of the value obtained for a water extract of soy meal. The pH 5.0 precipitate had only 5 mg TIA/g of meal. Further concentration of TIA in the supernatant by ultrafiltration in an Amicon cell was attempted. Most of the solids and activity stayed in the retentate and there was little additional concentration of TIA. Results of this procedure were compared with other procedures for fractionation of 2S, 7S, and 11S proteins. Better, more straightforward concentration of trypsin inhibitor was obtained by this procedure but separation of 7S and 11S proteins was not as good as with other methods.

- g. Specific Objective: Investigate pilot plant procedures for the separation of a trypsin inhibitor (TI) rich fraction from other components in soybeans.

Progress: Pilot-plant experimental studies are underway to develop procedures for separating a TI-rich fraction from the other components of defatted soy flour. To date, fractions with purities as high as 19% TI have been recovered, with the target purity being 26% TI.

- h. Specific Objective: Evaluate products from isopropanol (IPA) extraction of soybeans.

Progress: Sensory properties of oil and meal products from the IPA extraction of soybeans are being evaluated and indications are that both products exhibit properties very similar to those of the same products from a hexane extraction. Chick feeding trials are in progress using soybean meals from the IPA process (Cooperation with Mississippi State University). Soybean meals from the IPA process are also being evaluated in rumen by-pass studies in cooperation with the University of Illinois.

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4. Analytical and Structure Studies of Soybean Proteins (W. J. Wolf)

- a. Specific Objective: Determine if lipid oxidation disrupts membrane integrity and effect of additional membrane constituents on membrane structure.

Progress: The method chosen to prepare liposomes with a large internal aqueous space was that of Szoka and Papahadjopoulos (1978, Nat. Acad. Sci., USA, 75, 4194-4198) using a commercial source of soy phosphatidylcholine (PC). When formed in the presence of ^{14}C labeled sucrose, the liposomes entrapped 19 to 22% of the ^{14}C activity as determined by column chromatographic procedures, Ficoll density gradient sedimentation studies, and sedimentation pelleting at high centrifugal forces (100,000 g's). Continuous radio label monitoring of chromatographic column effluents indicates that soy PC liposomes interact to a significant extent with a variety of column polymers. A batch sequence of eluting solvents was needed to remove all of the ^{14}C activity even with columns pretreated with crude soy PC liposomes. This non-specific interaction was barely detectable using fresh egg yolk PC liposomes. GC analyses has clearly shown the differences in fatty acid composition between these lipid isolates.

Soy PC liposome systems containing ^{14}C sucrose have been maintained under air-saturated and nitrogen-saturated conditions at 30°C for 7 days. Nitrogen saturated controls were maintained at 4°C over this period. The UV spectra from 200 to 300 nm contain a single maximum at 206 nm for unoxidized liposomes (control) whereas the preparations maintained at 30°C had maxima at 206 and 234 nm. The ratio of A_{234}/A_{206} was 0.2 and 0.3 for the nitrogen saturated and air saturated systems, respectively, after 7 days. However, after this time, the extent of sucrose entrapment for the air saturated system had dropped from 19% to 15% while the liposomes maintained under nitrogen showed no loss of entrapped sucrose. After 3 days, only the air saturated system indicated a detectable 234 nm absorbance with no observable loss of encapsulated sucrose.

- b. Specific Objective: Study the aggregate-forming properties of β -conglycinin when treated with sonication and heat.

Progress: Comparative studies of sonic-induced soybean protein aggregates and aggregates produced by heating water extract at 80°C for 10 minutes showed differences in size and rate of formation. Gel filtration with Sepharose 2B and ultracentrifugation analyses of the aggregates indicated that sonication produced 30-50S species of aggregates versus 60-100S by heat, and the rate of formation by sonication was slower. Furthermore, the sonic-induced aggregates remained in solution while heat coagulated proteins precipitated readily. In a pilot-plant study of continuous ultrasonic extraction of soybean proteins, protein yield was found to be greatest at 1:30 soy-to-alkaline solvent ratio at 0.7 second of residence time. Energy input to produce protein isolate was the least at 1:10 soy-to-alkaline solvent.

- c. Specific Objective: Purify and characterize soybean calmodulin, and investigate interactions of calmodulin with soybean isoflavones

and study stimulation of soybean enzymes that require the presence of Ca^{+2} ions.

Progress: High-pressure liquid chromatography indicated the presence of extraneous contaminants. Purified soybean calmodulin showed a lower specific activity than bovine calmodulin and lower content of methionine per mole of protein.

- d. Specific Objective: Continue to investigate use of immunological techniques for determining amount of soy added to food products.

Progress: Partially purified β -conglycinin (7S) and glycinin have been prepared. Further purification is needed before the proteins can be injected into animals for the production of antibodies.

- e. Specific Objective: Determine the fate of toxic glycoalkaloids in soybeans contaminated with black nightshade (*Solanum ptycanthum*) seeds when processed into flours, concentrates, and isolates.

Progress: Glycoalkaloids from ground nightshade berries were extracted, flocculated by an ammonium hydroxide precipitation procedure, extracted, isolated by preparative TLC and characterized by HPLC, GC, and MS. Major glycoalkaloids found were β -solanoside, α -solanoside, and α -solanoside in about equal proportions, with lesser amounts of α -solanine and α -chaconine. A quantitative extraction procedure using methanol with nicotine as an internal standard was developed to extract the glycoalkaloids from nightshade, soy-nightshade blends, and fractions resulting when isolates and concentrates were prepared from soy-nightshade blends. The resulting extracts were analyzed by HPLC to determine individual and total glycoalkaloid content. With isolates and concentrates prepared by aqueous extraction from soy flours contaminated with nightshade, glycoalkaloids were distributed among all of the resulting fractions. When concentrates were prepared with 60% ethanol, however, glycoalkaloids were concentrated in the alcohol-soluble fraction and only very small amounts of glycoalkaloids occurred in the insoluble residue (concentrate).

- f. Specific Objective: Review literature on phosphorylation and deamidation of proteins as possible methods for changing protein functionality.

Progress: A review of the literature revealed that phosphorylation has been applied primarily to animal proteins such as egg albumin, hemoglobin, and dephosphorylated casein. Only one study (a U.S. patent issued to inventors in Taiwan) describes phosphorylation of soybean proteins. Conditions of phosphorylation (pH 11-12, 15-50°C for 2-3 hours), however, raise serious questions about nutritional safety because of the potential for formation of lysinoalanine. Several plant proteins including wheat gluten and zein from corn

have been deamidated by mild acid hydrolysis (0.02-0.5 N HCl at 100°C). The most noticeable effect on gluten and zein is an increase in solubility. The technique does not appear to have been applied to soybean proteins.

- g. Specific Objective: Participate in a review of the literature on bioavailability of iron from soybean and other proteins.

Progress: Bioavailability of iron from cereals and legumes, with emphasis on soybeans, was reviewed in participation with a task force of the International Anemia Consultative Group of the Nutrition Foundation, Inc. A report was issued which assesses the current situation of using soy protein as a meat extender and as a protein supplement in cereal-based diets. It was concluded that, generally, iron is poorly absorbed from cereals and legumes, but absorption can be enhanced by inclusion of meat and ascorbic acid in the diet. Substitution of meat with soy proteins as presently practiced poses few problems for those at low risk of iron deficiency (adult males and post menopausal women). Use of up to 30% substitution of meat with soy protein for infants, children, and women during childbearing years appears justifiable if there are adequate amounts of meat, fish, poultry, and ascorbic acid in the diet. Bioavailability of iron from cereal-soy blended foods is of concern because these products are normally used for feeding those at greatest nutritional risk. Use of stabilized ascorbic acid was recommended to help ensure availability of iron from these blended foods.

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5. Supercritical Fluid Technology for the Extraction of Seed Oils (J. P. Friedrich)

- a. Specific Objective: Study the solubility of free fatty acids and triglycerides in SC-CO₂.

Progress: Determination from PVT data by computing the internal pressure indicates that the overall solubility parameter of supercritical CO₂ in the range of 1000 to 5000 psi increases rapidly with pressure to 7-8 Hildebrand Units. At pressures much higher than 5000 psi, this computed solubility parameter tends to attain a constant value. These changes in solubility parameter parallel changes in density. In the range of 40°C to 80°C, the solubility parameter decreases with increasing temperature. Solubility parameters computed from the internal pressure average a little lower but are comparable to those computed from empirical equations (Bowman-Dense Gas Chromatography Studies-Ph.D. thesis, U. of Utah, 1976, p. 8-14).

From a study of the limited amount of published solubility data, straight chain C₁₂ to C₁₈ fatty acids have non-polar solubility parameters of about 9. The solubility parameters of ethanol, n-propanol and buteric acid computed from generalized PVT data are 8-10 and reach values of 17-18 at high pressure (15,000-20,000 psi). On the basis of non-polar solubility parameters, these materials as well as triglyceride would be expected to have limited solubility in supercritical CO₂. Previous reports by other researchers appear to bear this out. Solubilities of 1 to 3% triglyceride in SC-CO₂ were reported and the solubility curves tend to have the same shape as the solubility parameter and density curves for CO₂, i.e., they flatten out at high pressure.

Contrary to these observations and to theory based on present knowledge of solubility parameters, at temperatures above 60°C the solubility of seed oils increases dramatically with increasing pressure above 10,000 psi to well over 20%. This rapid response of solubility to temperature and/or pressure allows much faster extraction rates and affords more economical methods for recovery of the oil.

- b. Specific Objective: Extract with SC-CO₂ wet and dry milled corn germ, evaluate the oils and meals chemically and organoleptically, and develop technology to refine the oils.

Progress: Wet and dry milled crude corn oils from commerce were compared to oils obtained by SC-CO₂ extraction. The SC-CO₂ extracted crudes were lighter in color, lower in free fatty acids, phosphorus, and refining loss. Total unsaponifiables and tocopherols were about the same regardless of extraction method. Color reduction during laboratory processing of SC-CO₂ extracted oils paralleled that of the commercially extracted crude oils except that refined, bleached, and deodorized wet milled oil is lighter than that of the commercially processed oil.

Organoleptic evaluations of finished oils showed that SC-CO₂ extracted oils receive higher initial flavor scores than of hexane prepress or expeller oils. In addition, SC-CO₂ extracted oils possess superior flavor stability in 4 day aging tests.

Hexane extracted and expeller pressed crude oil contain phospholipids which act as antioxidants and protect them from oxidative deterioration during storage. Phospholipids show little solubility in SC-CO₂ and therefore these natural antioxidants are essentially absent and may be a disadvantage in long-term storage of SC-CO₂ extracted oils.

- c. Specific Objective: Develop an integrated process for soybean oil extraction with SC-CO₂ and physical refining; determine the effect of oxidation of crude oils on the quality of finished products; and study SC-CO₂ extraction of damaged beans.

Progress: SC-CO₂ extraction of damaged beans gives a much lighter colored oil than hexane extraction. Free fatty acids are high in both crude oils (2-4%) and phosphorous content is low (<100 ppm). Refining and organoleptic studies are being deferred until sufficient quantities of oil are available.

- d. Specific Objective: Determine the effect of moisture and configuration of beans and corn germs on the extraction of oil with SC-CO₂, and develop methodology for minor constituents in extracted oils.

Progress: In order to achieve residual oil levels of <0.01% it is necessary to flake to a thickness of <0.01" or to grind to flour of which 94% is less than 100 mesh. At a flake thickness of 0.015", 1.6% and at 0.023", 8.8% oil remains in the flake. Moisture has little bearing on extraction rate or efficiency except that at high CO₂ flow rates, the material will pack and plug the columns.

Phospholipids from crude soybean oil were separated by HPLC on a silicic acid column using a hexane:isopropanol:water solvent system. The lipid components were monitored by UV absorption at 206 nm. This proved to be a sensitive method of detection for phospholipids.

- e. Specific Objective: Cooperate with the ARS Southern Regional Research Center on the SC-CO₂ extraction of cottonseed to improve oil and meal quality.

Progress: Extraction of cottonseed with SC-CO₂ yields crude oils having improved properties compared to hexane prepress or expeller oils. Among these are markedly lower refining loss, phosphorus content and much lighter colors. Crude expeller and hexane prepress oils contain much higher levels of gossypol and related type pigments compared to SC-CO₂ extracted oils, i.e., 0.18 vs. 0.01% respectively. Unlike corn and soybeans where color is removed at all stages of processing, most of the color removal in cottonseed processing comes in the alkali refining step. Generally, this is accomplished by using an excess of caustic soda over that required to neutralize the free fatty acid. Laboratory refining studies showed that a SC-CO₂ extracted crude can be refined with 0.2% excess (10% lye) whereas a hexane prepress oil requires 0.5% for a similar color drop. For a plant refining 15 tank cars of oil/day (1.2% FFA), this amounts to a considerable savings on caustic soda costs. Analyses conducted at SRRC showed that gossypol in both hexane prepress and SC-CO₂ extracted oil is removed by caustic refining to levels below the detection limit.

- f. Specific Objective: Study lye requirements for crude soybean oils.

Progress: The literature contains relatively little information on the amount of lye required for caustic refining of soybean oils. Generally an excess above that required for neutralization of the free fatty acid is recommended because, in addition, phosphatide must be removed. Since the phosphatide content of SC-CO₂ extracted soybean oil is quite low, it would be less than that of a crude or degummed oil. Accordingly, a SC-CO₂ extracted soybean oil was refined with 10% lye at excesses of 0.05 and 0.1%, bleached with ½% activated clay and deodorized 3 hours at 210°C with citric acid. These oils had excellent flavor scores both initially and after 4 days storage at 60°C. Colors of the refined bleached deodorized oil were 7Y 0.3R. A crude degummed hexane extracted oil was also refined with 10% lye at 0.05 and 0.1% excess. As observed with SC-CO₂ extracted oils, these oils had excellent flavor scores and storage stability. Color of the RBD oils were 7Y 0.2R. These results indicate the lye requirements for a SC-CO₂ extracted crude are roughly the same as a degummed hexane extracted crude. CO₂ and hexane extracted oils refined under these conditions had phosphorus, and iron contents of >1 and 0.1 ppm respectively. These values are consistent with good quality soybean salad oils.

- g. Specific Objective: Extract wheat germ and bran with SC-CO₂ and evaluate products.

Progress: Wheat germ and bran were extracted with SC-CO₂ at 8000 psig and 50°C. The germ yielded 7% oil which was much lighter in color and had a milder odor than hexane extracted oil. Free fatty acid 8.9% was comparable to, unsaps (4.6%) less than, and tocopherol (4.5%) 1/3 greater than hexane extract. Tocopherol levels increased significantly in the final stages.

- h. Specific Objective: Evaluate supercritical CO₂-extracted soybean flakes.

Progress: Soybean flakes prepared by hexane extraction have been compared with soybean flakes prepared by supercritical carbon dioxide extraction. Various parameters such as flavor, nitrogen solubility, protein isolate yield, enzyme activity, amino acid analysis, and protein structure, have been evaluated and no significant difference noted. Cooperative research with Dr. M. Pubols, Washington State University has continued. Soybean meal samples generated during research into the feasibility of using supercritical CO₂ to extract oil from soybeans were fed to starting broiler chicks to determine their relative nutritive values. Pancreatic hypertrophy was evident in chicks consuming diets containing untoasted CO₂ extracted soybean meal. CO₂ extracted soybeans may provide a good protein source for the growing chick. The crude protein level of these preparations are comparable to commercially available hexane defatted soybean meal. A heat treatment appears to be necessary to inactivate the anti-nutritional factors in these meals similar to that required with hexane extracted meals.

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6. Phytic Acid Interactions and Iron Availability in Food Systems (Cooperative Agreement - Northwestern University)

- a. Specific Objective: Determine nature of phytic acid chelation with minerals.

Progress: Potentiometric as well as thermal titrations of phytic acid and its calcium complexes have been conducted using both the batch and titration microcalorimeters. For phytic acid, the experimental values by either method are in excellent agreement. For the calcium complexes, the total number of groups and the total heat evolved are in agreement but the placement of the curves is different due primarily to the differences in calcium concentration.

Binding of calcium by phytic acid is endothermic for the pH range 2-11 while the heat of dilution of 1 M CaCl_2 is exothermic. The binding at pH 11.16 gives a value of 19.6 kcal/mole. Inspection of the thermal binding curve indicates that a number of step binding constants are involved and that 5.3 moles calcium are bound per mole phytic acid. This value has been confirmed by atomic absorption spectroscopy. Both the thermal and the potentiometric curves are reversible either by the instant injection of acid or base or by continuous titration. Values for the ionization constants (as pK') and the enthalpy of ionization (as ΔH_i) have been estimated by computer assisted curve-fitting. These results are the necessary prerequisite data for the study of phytic acid and metal binding to protein systems.

7. Effects of Dietary Soy Protein on Metabolic Processes in Subhuman Primates (Cooperative Agreement - New England Regional Primate Research Center)

- a. Specific Objective: Develop protocols for the measurement of effects of long-term feeding of diets containing soy proteins on the pancreatic status of cebus monkeys.

Progress: This cooperative agreement was initiated October 1, 1982. A coordinative meeting involving ARS, FDA and cooperating scientists was held at the Primate Center, Southborough, Massachusetts, November 2 and 3, 1982. Facets to be included in the protocol are: Maintenance of the 27 cebus monkeys on the current diets. Tests to establish the current pancreatic status of the cebus monkeys: Non-invasive tests [PABA Peptide, Fecal Enzyme, Blood Hormone, GI Secretory, CAT SCAN (tentative)] and Biopsy tests (RNA, Protein, Enzyme, Pathology/Histology, Photograph of Pancreas at time of biopsy for estimation of size). Inclusion of clinical history of monkeys, detailed results of observations during six month test period and available information during subsequent maintenance period. Additionally, availability of autopsy data on deceased monkeys will be helpful in interpretation of data accumulated on the test monkeys.

B. BIOMATERIALS SCIENCE

1. Basic Chemistry of Vegetable Oils for Fuels and Alternative Chemicals (E. H. Pryde)

- a. Specific Objective: Develop and determine the physical properties of aqueous ethanol/vegetable oil microemulsions for the purpose of lowering viscosity and improving combustion properties of these hybrid fuels in farm tractor engines.

Progress: Both detergent and detergentless microemulsions were prepared. The detergentless microemulsion is a hybrid fuel in which aqueous ethanol is dispersed in the vegetable oils with 1-butanol serving as a single-component nonionic surfactant. Rheology, ultracentrifugation, conductivity, refractive index, and density determinations support the microemulsion model of an alternative structure in which oil-rich and water-rich domains are randomly interspersed at equilibrium.

- b. Specific Objective: In cooperation with Professor Carroll Goering at the University of Illinois, determine the performance of both detergent and detergentless microemulsions in short-term engine tests. These tests are preliminary to longer-term, endurance tests to be carried out at the Allis-Chalmers Engine Test Laboratory in Harvey, Illinois.

Progress: A short term engine evaluation at the University of Illinois with a "John Deere Model 152" power unit showed that although the detergentless microemulsion contained 19% less energy per kilogram than No. 2 diesel fuel, it produced almost the same peak power. It was noted that the detergentless microemulsion burned cleaner than diesel fuel, and diesel knock was comparable for the two fuels. Thus, the lower cetane number for the microemulsion was not reflected in engine performance. Brake specific fuel consumption was somewhat higher for the hybrid fuel than with diesel fuel. The detergent microemulsion was formulated with a surfactant system prepared from equimolar quantities of soybean oil acids and triethylamine. A co-surfactant (1-butanol) was also used in this system to obtain the desired viscosity. This fuel was tested in a "John Deere Model 152" power unit. Although the ionic hybrid contained 20% less energy per kilogram than No. 2 diesel fuel, it produced almost the same peak power. This hybrid fuel burned cleaner than diesel fuel, and performance was generally similar to that of the detergentless microemulsion. Longer-term engine tests according to the Engine Manufacturer's Association recommended procedures have been conducted at the Allis-Chalmers engine test laboratory in Harvey, Illinois, and at the University of Illinois, Urbana. (See Northern Agricultural Energy Center, A.6.)

- c. Specific Objective: Continue to assist in the transfer of technology from the laboratory to interested parties in the development of petroleum-sparing alternative chemicals and fuels for oilseed crops.

Progress: A review on the current state of the art on commercial vegetable oils as alternative chemicals was presented at the American Association for the Advancement of Science Annual Meeting in Washington, D.C., in January (see Northern Agricultural Energy

Center, A.5.). A symposium on Nonfood Uses of Plant Oils was organized for the American Oil Chemists' Society Annual Meeting in Toronto, Canada, May 1982. A symposium is being organized for the next AOCS meeting in May 1983, on the use of vegetable oils as replacements for petroleum oils as carriers for herbicides and insecticides used in crop production. Planning was continued for the session on new chemistry of fatty acids at the AOCS Oleochemicals International Conference in 1983.

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2. Physical/Chemical Modification of Vegetable Oils for Diesel Fuel (E. H. Pryde)

See Northern Agricultural Energy Center, A.5.

3. Long-Term and Endurance Engine Tests with Vegetable Oil Products as Diesel Fuel (Cooperative Agreement - North Dakota State University)

See Northern Agricultural Energy Center, A.6.

4. Production Problems of the Oilseed Crop, Crambe (Crambe abyssinica) (Cooperative Agreement - Murray State University)

See Northern Agricultural Energy Center, A.8.

C. TECHNOLOGIES AND PRODUCTS TO INCREASE EXPORTS OF AGRICULTURAL PRODUCTS

1. High-Temperature Soybean Cooking Oils for the Export Markets (E. N. Frankel)

- a. Specific Objective: Optimize continuous slurry high pressure system for selective hydrogenation of soybean oil with copper-chromite catalyst to improve technology for soybean oil products in export markets.

Progress: Soybean oil was hydrogenated continuously with copper-chromite catalyst in a 120 ft tubular reactor. A series of hydrogenations was performed according to a statistical design varying processing conditions such as oil flow (0.5, 1.0, and 2.0 l/hr), reaction temperature (180 and 200°C), hydrogen pressure (1,100 and 4,500 psi) and catalyst concentration (0.5 and 1.0%). Analysis of variance showed that linolenate selectivity and trans isomerization were not affected by change in pressure, temperature, or catalyst concentration. As expected, increase in temperature, pressure, or catalyst concentration resulted in increased hydrogenation. Increasing the oil flow rate at 1,100 psi gave higher reaction rates possibly because of increased turbulence

within the reactor. At the higher pressure, hydrogenation increased when flow rate was increased from 0.5 to 1.0 l/hr. To hydrogenate large volumes of vegetable oils at high pressure, continuous reaction with a selective catalyst such as copper-chromite may offer economic advantages over batch hydrogenation.

- b. Specific Objective: Develop a continuous stationary catalyst bed hydrogenation process to improve the present technology to produce soy food products for domestic and export markets.

Progress: A mini-stationary catalyst bed reactor was designed, constructed, and operated to study the effect of operational parameters on the selective hydrogenation of soybean oil. Palladium on carbon and on polymer and nickel catalyzed the hydrogenation of soybean oil with low selectivity. Soybean oil was hydrogenated at liquid hourly space velocities (LHSV) from 5 to 20; pressures from 20-50 psig; and temperatures from 120 to 260°C. While the linolenate selectivity for all runs were nearly the same (1-2), the linolenate selectivity, conjugated diene, trans isomers, and extent of hydrogenation increased by increasing temperature from 220 to 260°C.

The Berty (fixed basket catalytic) reactor was operated in a batch mode in an attempt to evaluate catalysts under gradientless conditions. Use of Pd on 1/8" alumina pellets catalyst (0.07% Pd in soybean oil) at 100-120°C, 50 psig and agitator speed of 1,000 rpm selectivity (SR=3) reduced the linolenate from 7.5% to 0.7% in 20 min. Shorter reaction time produced much less linolenate reduction.

- c. Specific Objective: Relate volatile profiles produced by gas chromatography (GC) with sensory evaluations of soybean oils aged under different conditions.

Progress: A direct GC analysis of volatiles was applied to measure the flavor stability of soybean oils after aging at 60°C. Prominent peaks identified by GC-MS included: pentane, hexanal, 2-heptenal, 2,4-heptadienal, 2-decenal, and 2,4-decadienal. Induction periods calculated for the formation of volatiles showed the oils with the tertiary butylhydroquinone (TBHQ) + citric acid combination, with the citric acid only and with no additives to have inductions of 9, 5, and 0 days, respectively, for total volatiles. Pentane, 2,4-decadienal, and total volatiles were most related to deteriorative changes and produced characteristic induction curves. Correlation coefficients of volatiles with flavor scores were also high. This study showed that flavor stability can be predicted by determining induction periods based on GC volatiles analysis and such determinations can complement sensory evaluations.

Eight different vegetable oils obtained commercially were analyzed for volatiles by capillary gas chromatography. Volatiles generated in a gas chromatography (GC) headspace sampler were automatically injected onto a 30 m capillary column. Only a small number of volatiles of low intensity were present in the fresh samples which varied in peroxide values from 1 to 3. Several major peaks were evident in the oils aged 8 days at 60°C with peroxide values ranging from 5 to 30. Identification of the GC peaks was tentatively based on the relative retention time of reference compounds and by GC-MS. Major volatiles common in each of the vegetable oils included butane, pentane, hexanal, heptenal, and 2,4-decadienal.

- d. Specific Objective: Improve sensory evaluation scoring procedure by more direct computer data handling.

Progress: Procedures were developed to permit taste panel members to directly enter their sensory scores and descriptions onto 80-column computer cards using voting machines. Computer programs were written to allow immediate calculations and statistical analyses of data. Examination of data from previous tests using scoresheets showed no difference in scores with those from the voting machines. Significant savings in time for data handling and decrease in errors from transfer of data has resulted.

- e. Specific Objective: Evaluate commercial oils for isomers formed during processing.

Progress: Deodorized soybean oil samples were obtained from several commercial oil processors under different conditions used for deodorization. Analysis by capillary GC indicated that small amounts of trans containing diene and triene isomers were formed in each sample. 0.1%-0.4% of mono-trans dienes and di-trans trienes were present in each deodorized oil. Refined and bleached soybean oil obtained from one of the oil processors was deodorized in the laboratory. Temperature ranged from 210°C to 290°C and vacuum varied from 1 mm to 15 mm mercury. Diene and triene isomers containing trans were not present in the laboratory deodorized samples.

- f. Specific Objective: Determine the feasibility of converting soybean oil to cooking and salad oils with the use of ultrasonic energy.

Progress: The rate of hydrogenation of soybean oil with either copper-chromite or nickel catalysts increased more than a hundredfold with the aid of ultrasonication. With ultrasonic energy, 87% hydrogenation of linolenate in soybean oil was obtained in 8.6 seconds at 115 psig H₂ with 1% copper-chromite at 181°C and 77% hydrogenation with 0.025% nickel. Without ultrasonic energy only 59% linolenate hydrogenation was obtained in 240 seconds with

copper-chromite at 198°C and 500 psig H₂ and 68% linolenate hydrogenation in 480 seconds with nickel at 200°C and 115 psig H₂. In a continuous reaction system the selectivity with copper-chromite catalyst for linolenate reduction was lower when ultrasonic energy was applied. This innovation may offer an important advantage in increasing the activity of commercial catalysts for fats and oil hydrogenation particularly for copper-chromite.

- g. Specific Objective: Identification of volatiles from heated (192°C) tri (cis-9,cis-15-octadecadienyl) glycerol.

Progress: GC data shows twelve primary volatiles are formed upon oxidation of isotrilinolein. Of those compounds, ethanal, propanal, 2-butenal, 2-pentenal, 6-nonenal, and 3-heptene have been identified. Mass spectra of the remaining six primary compounds indicate they include 5-octenal, 2,7-decadienal, and 2,8-undecadienal. Authentic aldehydes are required to corroborate the tentative identification of those latter substituents.

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2. Quality Soybean Oil for Export Markets (E. N. Frankel)

- a. Specific Objective: Evaluate quality of crude oil from soybeans damaged during storage.

Progress: Five experiments were completed in the small adiabatic reactor. Bean moisture concentration ranged from 17- $\frac{1}{2}$ % to 19- $\frac{1}{2}$ %. Maximum intrinsic temperature varied between 46.8°C and 51.9°C and reaction time from 3 weeks to 13 weeks. Poor placement of temperature sensor in reactor for first 3 runs caused lower intrinsic temperature reading but should have little effect on the extent of damage. Five analyses were run on the hexane-extracted oil from the damaged beans, peroxide value, percent and composition of free fatty acid (FFA), and iron and phosphorus concentration. FFA composition varied little with storage damage (with a possible trend toward lower linolenic acid) and FFA percentage was high as expected. Phosphorus concentrations were very low, ranging from '4 to 80 ppm.

- b. Specific Objective: Establish experimental conditions for the removal of phosphatides in soybean oil with surface active agents.

Progress: Aqueous degummings of oils from properly-stored and damaged soybeans were made with a series of 5 anionic, 2 cationic, and 4 nonionic detergents, and crude lecithin as an amphoteric emulsifier. Efficiency of phosphatide removal was determined by analyzing the degummed oils for phosphorus colorimetrically. Nonflame atomic absorption analysis was sufficiently sensitive only for the high levels of phosphorus in degummed oils from damaged beans. For normal oils, efficient degumming was primarily the result of citric acid addition. This efficiency was improved, however, by crude lecithin, sodium dodecyl sulfate addition, and fatty oxazoline. Weakly and strongly ionized anionic and cationic surfactants and some nonionic agents were without effect, whereas, high molecular weight nonionics hindered efficient degumming.

Degumming of oils from damaged beans indicated high levels of nonhydratable phosphatides with were little affected by additives. Although nonionic high-molecular weight and anionic surfactants in combination with citric acid increased the efficiency of degumming damaged oils, crude lecithin in similar application did not degum the oil and was quantitatively retained as nonhydratable phosphorus.

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3. Heavy Metals in Soybeans Grown on Sewage Sludge-Amended Soil (Cooperative Agreement - Western Illinois University)

- a. Specific Objective: Investigate the effect on soybean yield and the modification of trace metal content of soybeans, meal, and oil from the use of municipal sewage sludge for fertilization.

Progress: Scientists at Western Illinois University have completed the third year of growing experimental plots of soybeans on sludge-treated soils. The plots on which a third year annual application

of sludge was made had an unusually thick growth of Kochia weeds which severely reduced yields. This weed is not normally associated with soybean plots and was assumed to arise from the source of sludge. Other plots without current year sludge application gave yields equal to or better than previous year. This is the final planting. Analysis of soil and interpretation of results will be completed prior to termination date of June 1983.

D. HUMAN REQUIREMENTS FOR NUTRIENTS

1. Biochemical Behavior of Isomeric Fats in Hydrogenated Soybean Oil (E. A. Emken)

- a. Specific Objective: Interpret data and prepare manuscript on the incorporation of trans-11- and cis-11-octadecenoic acid in human blood lipids.

Progress: Blood samples from two human subjects fed a mixture of deuterated cis-9-, trans-11- and cis-11-octadecenoic acid were separated and derivatized for GC-MS analysis. Nearly 600 triglyceride, cholesteryl ester and various phospholipid fractions from plasma, red blood cell, chylomicron, very low density, low density and high density lipoprotein samples were isolated. Emphasis on GC-MS analysis of samples from studies outlined in objectives b and d were accelerated because of unexpected developments and forced the analysis of these samples to be postponed. This work is in cooperation with Dr. Gully, St. Francis Medical Center, Peoria, Illinois.

- b. Specific Objective: Demonstrate feasibility of a multiple deuterium isotope technique for simultaneously comparing metabolism of 5 different fatty acids.

Progress: A mixture of deuterated 16:0, 18:0, 9c-18:1, 9c, 12c-18:2, and 12c, 15t-18:2 was fed to a young adult male subject. The fatty acids were fed as their triglycerides and plasma and urine samples were collected. Triglyceride, cholesteryl ester, free fatty acid, and phospholipids were isolated from total plasma, chylomicron, VLDL, LDL and HDL samples. GC-MS analysis by Dr. Ferretti, ARS Lipid Nutrition Laboratory, Beltsville, MD, of prostaglandin metabolites isolated from urine samples did not detect deuterated prostaglandin metabolites resulting from conversion of 9c, 12c-18:2-d₄ to prostaglandin via the cyclooxygenase pathway. GC-MS analysis of plasma and lipoprotein lipids is nearly complete and the data is in the process of being interpreted.

- c. Specific Objective: Initiate exploratory experiments on the effect of fatty acid isomers on human platelet membrane function.

Progress: Human platelets obtained from the American Red Cross Blood Unit were incubated with various fatty acids and the rate of aggregation followed. Due to changes in Red Cross regulations, only outdated platelets are now available which were found to give widely variable results. Procedures were subsequently explored for obtaining platelets from rats and experiments initiated to confirm validity of platelet aggregation techniques and reproducibility of data.

- d. Specific Objective: Initiate research on determination of metabolic fate in humans of trans- and cis-10-octadecenoic acid isomers present in hydrogenated soybean oil.

Progress: Mixtures of triglycerides containing deuterium labeled trans- and cis-10-octadecenoic acids and cis-9-octadecenoic acid were administered orally to two young adult male subjects. Nine blood samples were collected during a 48 hr period and plasma, red blood cells and lipoprotein (chylomicron, VLDL, LDL, HDL) fractions were isolated. Lipid from each fraction was separated by thin layer chromatography into triglyceride, cholesteryl ester, free fatty acid, phosphatidylcholine, phosphatidyl ethanolamine, phosphatidyl serine, sphingomyelin, and lysophosphatidyl choline. Samples from one subject have been analyzed by GC-MS and the data is the process of being evaluated.

- e. Specific Objective: Continue fatty acid adsorption studies in patients with cystic fibrosis.

Progress: Mixtures of deuterated stearic or palmitic acid, elaidic acid, oleic acid, and linoleic acid were used in a multiple labeled study. Emulsions of the fatty acids as their bile salts were administered to 2 subjects via a stomach tube. Samples of plasma and intestinal aspirates are in the process of being separated and derivatized for analysis by gas chromatography-mass spectrometry.

Modification of Dr. Parson's mass spectrometer and analytical methodology was suggested by NRRC personnel in order to increase the sensitivity sufficiently to permit more accurate analysis of the samples. This work is in cooperation with Dr. Parson, University of Calgary and Dr. Kuksis, University of Toronto, Canada.

- f. Specific Objective: Initiate study to investigate the effect of dietary fats on human milk lipid composition.

Progress: A protocol patterned after NRRC studies was approved to allow 3 g each of deuterated 16:0, 18:1 and 18:2 to be fed to a lactating subject. The triglycerides of these deuterated fats were prepared at NRRC and fed to a mother who had been breast feeding her infant for 5½ months. Milk, plasma and lipoprotein blood samples were obtained periodically for 72 hr after feeding.

Extraction and separation of the lipoprotein lipids has been initiated at NRRC. Work is in cooperation with Dr. Garza and Dr. Hachey, Baylor College of Medicine and Children's Nutrition Research Center, Houston, Texas.

- g. Specific Objective: Explore separation and characterization of the geometric isomers of methyl 9,12,15-octadecatrienoate.

Progress: The four binary mixtures of the geometrically isomeric methyl 9,12,15-octadecatrienoates were separated by partial silver resin chromatography and the eight pure methyl esters obtained were characterized. Physical constants determined included melting points, percent trans by infrared, equivalent chain lengths and ^{13}C nuclear magnetic resonance chemical shifts.

- h. Specific Objective: Devise an improved scheme for obtaining pure methyl dihydroxyoleate from the mixed Vernonia esters.

Progress: It was determined that reverse phase high pressure liquid chromatography using a Waters Associates Prep Pak 500/C18 column with the solvent system acetonitrile:water (90:10) provided a simple and rapid method for separating substantial quantities of methyl dihydroxyoleate from the non-hydroxy esters present in Vernonia methyl esters.

- i. Specific Objective: Complete the syntheses of methyl 9,12-octadecadienoate- d_4 isomers.

Progress: The four geometrically isomeric methyl 9,12-octadecadienoates-15,15,16,16- d_4 were prepared as two binary mixtures which were separated by partial silver resin chromatography. Equivalent chain lengths and melting points were determined.

- j. Specific Objective: Prepare a review article on synthesis of deuterated fatty acids.

Progress: Upon the invitation of Dr. Ralph Holman, Editor of Progress in Lipid Research, Dr. Rakoff prepared a review article on the synthesis of deuterated fatty acids and esters which recently appeared in print.

- k. Specific Objective: Present at an invited symposium at the AOCS meeting in Toronto in May 1982, a review of our synthetic work on the preparation of mono, di, and trienoic esters containing a 12,13-double bond.

Progress: A paper entitled Synthesis and Characterization of All the Geometrically Isomeric Methyl 9,12- and 12,15-Octadecadienoates and 9,12,15-Octadecatrienoates was presented at the meeting. A manuscript entitled Syntheses and Properties of Mono-, Di-, and

Trienoic Fatty Esters Containing a 12,13-Double Bond has been accepted by Journal of the American Oil Chemists' Society.

- l. Specific Objective: Study various methods for the separation and purification of triglycerides composed of octadecadienoic and octadecatrienoic isomers.

Progress: Completed evaluation of synthetic procedure for using the sodium methoxide catalysed interesterification of methyl linolenate with triacetin to prepare trilinolenin. Yields (~70%) are better than the coupling of the acid chloride and glycerol. This procedure also produces less isomeric by-products than the p-toluenesulfonic acid catalysed esterification of glycerol by linolenic acid (higher temperature required). Multigram quantities of triglycerides (prepared by the interesterification of triacetin and the appropriate methyl ester) were purified by silver ion chromatography. A 47 X 400 mm glass column packed with silver-saturated XN1010 resin was used to separate the methyl esters from the triglycerides. The solvents used were 100% acetone for oleate, 8.5% acetonitrile in acetone for linoleate and 15% acetonitrile in acetone for linolenate.

- m. Specific Objective: Synthesize deuterium labelled fats for DSC analyses to determine effect of the deuterium atoms on spacial orientation and thermodynamic properties.

Progress: Continued cooperative project with Horticultural and Special Crops Laboratory. Provided Dr. J. Rothfus and Dr. S. Chang with samples of linoleic acid 15,15,16,16-d₄; -16,16,17,17-d₄, and 17,17,18,18-d₄ for DSC analysis.

- n. Specific Objective: Evaluate catalytic methods for the reduction of polyunsaturated fatty acid esters.

Progress: The Lindlar (palladium on calcium carbonate, poisoned with 5% lead) reduction (D₂ gas) of methyl stearoleate to methyl oleate-9,10-d₂ was studied. Various poisons were utilized (quinoline, quinaldine, acridine, and pyridine) and the product was analyzed for over-reduction and deuterium scatter. Except for acridine, the others were all found to be satisfactory. Isotopic purity of the oleate-9,10-d₂ was 94%.

- o. Specific Objective: Prepare (Z,Z)- and (E,Z)-3,5-tetradecadienoic acids which are sex attractants for Attagenus elongatulus and Attagenus megatoma, respectively.

Progress: Coupling of a 1-bromo acetylene with an acetylenic alcohol gave 3,5-tetradecadiyn-1-ol which after reduction and oxidation gave (Z,Z)-3,5-tetradecadienoic acid in overall 18% yield. Coupling of a terminal acetylene with methyl 4-bromo-2-

butenoic acid gave largely methyl (Z)-3-tetradecen-5-ynoate which was reduced to methyl (Z,Z)-3,5-tetradecadienoate. The (E,Z)-tetradecadienoic acid can be obtained only by non-acetylenic coupling of appropriate intermediates followed by separation, reduction, and oxidation.

- p. Specific Objective: Improve and modify the mass spectrometers to increase their suitability for the analysis of high mass low volatility compounds.

Progress: A special chemical desorption probe was designed and made for use with ammonia chemical ionization and the Finnigan mass spectrometer to determine the molecular weight of several solasodine triglyccosides.

The high resolution mass spectrometer was tested out to a mass accuracy of 3 millimass using GCMS computer techniques and used to measure unknowns isolated from fermentation products.

- q. Specific Objective: Develop new techniques to analyze blood lipids of subjects fed deuterium labeled linoleic acid isomers plus 4 deuterium labeled internal standards or other dietary fatty acids.

Progress: New methods using isobutane chemical ionization and fused silica capillary columns have been developed for analysis of labeled blood lipids on the Finnigan mass spectrometer. The old method measured only methyl oleate and its deuterated analogues while the new method measures all the C₁₆, C₁₇ (added as an internal standard), C₁₈, and C₂₀ methyl esters along with any labeled analogues fed or generated during metabolism. This new technique generates a vast amount of data which requires that special computer programs be developed to produce a useful output.

- r. Specific Objective: Identify molecular structures using gas chromatography-mass spectrometry and other methods in collaboration with scientists within NRRC.

Progress: Several studies of trichothecenes in contaminated farm products have been analyzed by GCMS and the mass spectrometer data continues to make a vital contribution to the studies of oxidation of lipids.

- s. Specific Objective: Complete feeding studies of trans-10- and cis-9-octadecenoic acids to laying hens.

Progress: Analyses of major lipid classes from egg yolks and tissues of laying hens after feeding trans-10-octadecenoate-³H (10t-18:1-³H) and cis-9-octadecenoate-¹⁴C (9c-18:1-¹⁴C) have been completed. Preferential incorporation of 9c-18:1-¹⁴C occurred in

the octadecenoate component of all lipid classes examined (triglycerides, cholesteryl esters, phosphatidylethanolamines, and phosphatidylcholines). Chain shortened and novel desaturated metabolites arising from the $10t-18:1-^3H$ contained from 20% to 45% of the tritium label recovered in the major egg yolk lipids. The 1-acyl position of phosphatidylethanolamine was the only lipid fraction to show greater incorporation of $10t-18:1-^3H$.

Tissue lipid analyses indicated that there was preferential deposition of $9c-18:1-^{14}C$ into all organs. Heart tissue exhibited the largest discrimination against $10t-18:1-^3H$. Individual liver lipid classes displayed the same relative order of discrimination against $10t-18:1-^3H$ as did egg yolk lipids. Chain shortened and desaturated metabolites from $10t-18:1-^3H$ were also detected in liver lipids.

- t. Specific Objective: Explore the possibility of separating pure arachidonic acid from its geometrical isomers.

Progress: A rapid and effective method for isolation of pure methyl arachidonate from its geometric isomers and from reaction products formed during syntheses has been developed. Using a mixed solvent system of acetonitrile/methanol in conjunction with a column packed with a sulfonic acid resin fully saturated with Ag^+ ions, pure methyl arachidonate was obtained on a semi-preparative scale in 40 minutes.

- u. Specific Objective: Determine cutaneous permeability of the pesticide parathion when starch encapsulated.

Progress: ^{35}S -Parathion was encapsulated in a starch xanthate matrix. Radioactive incorporation was 93% to give a specific activity of .006 $\mu ci/mg$. This encapsulated material was sent to Dr. Ronald Riley at the Russell Research Center where the relative safety of starch-xanthate encapsulated parathion was compared to parathion adsorbed on clay granules.

Compared to adsorbed parathion, encapsulation of parathion slowed release of the pesticide into aqueous solvents (perspiration) and air, and also reduced cutaneous penetration and accumulation. Encapsulation is an improved formulation for safe handling. This work was done in cooperation with F. Otey and B. Shasha, Biomaterials Conversion Laboratory.

- v. Specific Objective: Complete determination of distribution of dietary octadecenoic acid isomers in individual lipid classes in human tissues.

Progress: The level of trans-18:1 isomers in several isolated lipid classes of human liver, heart, red blood cells and plasma was determined. Phospholipids contained substantially less trans-18:1 isomers than triglycerides. The double bond distribution of the cis and trans octadecenoate fraction of triglycerides and phosphatidylcholine from human liver and heart was determined. Whereas the double bond distribution of the triglycerides correlated closely with the pattern found in dietary hydrogenated vegetable oils, the phosphatidylcholine fraction showed evidence of selective incorporation or metabolism of specific trans positional isomers. In general, isomers with double bonds near the methyl terminus were present at levels higher than expected from their relative abundance in the diet.

- w. Specific Objective: Evaluate various methods for the measurement of rates of fatty acid oxidation by human heart tissue.

Progress: A source of fresh human heart tissue has been arranged through cooperation with the cardiac surgery department of St. Francis hospital. The technology needed for preparing viable individual human heart cells was found to be inadequate for optimum fatty acid oxidation experiments. An alternative method using heart tissue homogenates has been tested and found to give high rates of fatty acid oxidation. Uniformly labelled elaidic acid has been prepared and its rate of oxidation compared to oleic acid using tissue from both rats and humans.

- x. Specific Objective: Evaluate possible use of lipid oxidants as a negative selection procedure to allow isolation of soybean mutants with reduced linolenic acid content.

Progress: When seeds of soybeans or safflower were stored under high oxygen concentrations, their percent germination declined rapidly. For example, soybean seeds stored under 7.7 atmospheres oxygen pressure at 25 C and 17% moisture lost all viability within 22 days. Germination decreased continually in oxygen pressures ranging from 0 to 7.7 atmospheres. High levels of oxygen, moisture, or temperature each acted independently to cause losses of germination, but when applied simultaneously, these factors acted synergistically. Soybean seeds were also aged under conditions of high temperature (44 C) and humidity (100% RH), which have been routinely used to accelerate aging. Under these conditions, no oxygen dependence of seed death was observable.

Increased lipid oxidation was not detected in seeds that had lost germination ability due to high oxygen treatment. Seeds of two safflower varieties that contained either high oleic or high linoleic fatty acid compositions were subjected to high oxygen treatment. Although the lipid of the high oleic variety is markedly more stable to oxidative degradation, we detected no significant difference in the oxygen tolerance of these seeds.

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NORTHERN AGRICULTURAL ENERGY CENTER

M. O. Bagby, Manager

A. BIOMATERIALS SCIENCE

1. Hydrocarbon-Producing Plants as Potential Multi-Use Crops (M. E. Carr)

- a. Specific Objective: Identify and call attention to plant species that are potentially adaptable to U.S. agricultural practice and have an overall composition favoring their economic development as multi-use botanochemical-producing crops.

Progress: Plant scientists have been solicited for collecting whole plant samples of 900 species from Oregon, Arizona, North Dakota, Colorado, Tennessee, Indiana, Ohio, and Illinois. Samples of 250 species and voucher specimens have been received. Other cooperating scientists have provided 20 species from Florida and Arizona. An additional 100 species from central Illinois have been collected by NRRC scientists. Whole plant samples of 141 species which were collected in 1981 and 1982 by NRRC and cooperating collectors, have been analyzed and evaluated for their potential as oil- and hydrocarbon-producing species. There were 28 species considered promising with NRRC ratings of 8-11 based on their botanical characteristics and contents of oil, hydrocarbon, and apparent protein. Extracted from these promising species were 2-7% oil, (dry whole plant weight basis) and 0.01-1.8% hydrocarbon. Polyphenol fractions, which were removed with the oil in acetone extractions, were 4-21%. Promising species and their ratings include, for example, Asclepias syriaca L. (common milkweed, 8), Isocoma drummondii (goldenweed, 10), Arctium lappa L. (Burdock, 10), Rhus aromatica Ait. (fragrant sumac, 11), Quercus rubra L. (Red oak, 11), Spirea bumalda Bury (Spirea, 11), and Cacalia atriplicifolia L. (Pale Indian plantain, 11).

- b. Specific Objective: Characterize oils, polyphenols, and hydrocarbon polymers produced by select U.S. plant species.

Progress: The oil obtained from acetone extractives of 15 species were characterized with respect to TLC, unsaponifiable matter, and free acids of the saponified oil. Hydrocarbon (hexane extractives) was characterized by IR spectroscopy and GPC. Acetone extractives of whole plant Asclepias syriaca L. (common milkweed) which were immediately freeze dried after collection, contained an average of about 18% acetone extractives (dry plant weight basis) compared to about 9% for samples allowed to dry and age 3 to 4 months at room temperature conditions. Analyses of the acetone and hexane extractives of the milkweed samples revealed major quantitative differences were in the amounts of polyphenol fractions extracted

from the samples rather than in the amounts of oil and hydrocarbon. Saponification of the oil fractions yielded 66 and 76% unsaponifiable matter and 12.4 and 16.5% free fatty acids from the air- and freeze-dried samples, respectively. Average molecular weight of the hydrocarbons was about one-sixth of the Hevea tree. A more detailed characterization study was conducted of the oil from freeze-dried milkweed using techniques including TLC, CC, HPLC, GC, IR, and MS. This study revealed that 46% of the whole-plant oil fraction was comprised of lupeol and amyrin (alpha and beta), which are pentacyclic triterpenes.

- c. Specific Objective: Cooperate with university and USDA scientists in plant-breeding efforts to develop biomass crop varieties.

Progress: In cooperative work with USDA agronomists from Georgia and Maryland, 140 samples of Rhus glabra and Rhus copallina (sumac) and 152 samples of Ipomoea batatas L. vines (sweet potato) were analyzed for acetone and hexane extractives. Sumac stem samples had a range of 8-24% total acetone extractives (dry stem weight basis), which contained oil fractions of 1-3% and polyphenol fractions of 6-12%. Sumac leaves had 27-66% acetone extractives (dry leaves weight basis) containing 6-10% oil and 21-48% polyphenol fractions. Hexane extractives (hydrocarbon) were 0-0.44% in the stems and 0-0.83% in the leaves. In sweet potato vines the total acetone and hexane extractives were about 3-15% (not partitioned into oil and polyphenol fractions) and 0-0.5% hydrocarbon, respectively. Recently 75 samples of Asclepias syriaca (mostly leaves) have been received from Maryland for compositional analyses in a cooperative plant-breeding heritability study.

In cooperative sugar crop production efforts with 12 USDA collaborators about 1700 samples of corn, sorghum, and beets have been analyzed. Samples analyzed for glucose, sucrose, and fructose included corn (1372), sorghum (226), and beets (70). Samples analyzed for lignin were corn (309) and sorghum (136) and for cellulose were corn (401) and sorghum (136).

- d. Specific Objective: Evaluate various plant resins, oils, hydrocarbons for potential industrial utility.

Progress: Four species previously identified as having potential quantities of high energy extractables have been collected in 25-lb quantities and submitted to a university cooperator for a gasification and biomass quality study.

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2. Microbiological and Enzymatic Procedures for Conversion of Agricultural Residues and Biomass (R. W. Detroy)

- a. Increase efficiency of saccharification of lignocellulosic residues to fermentable sugar.

Progress: Thermal hydropulping (an autohydrolysis process) was effective for selectively removing and recovering hemicellulose (pentosan)-rich fractions from wheat straw (WS). This pretreatment increased the saccharification efficiency of the WS residues treated with cellulase. After solvent extraction and subsequent acid hydrolysis of the pentosan-rich fraction to primarily xylose, a study was conducted on the fermentability of the crude xylose to ethanol by *Pachysolen tannophilus*.

In the thermal hydropulping study chopped WS was pressure cooked at 170, 180, and 190°C for 30 min. Alpha cellulose contents of the pulps decreased slightly with increasing pulping temperatures above 170°C (e.g., 2% at 180°C and 11% at 190°C). Maximum liquor solids were realized from the 180°C cook. By increasing pulping temperatures from 170°C to 185°C, little change in lignin contents of the pulps occurred. Removal of pentosan, however, increased from 62% at 170°C to 94% at 185°C.

Agricultural straw was subjected to thermal or alkali pulping prior to enzymatic saccharification. When wheat straw (WS) was treated at 170°C for 30-60 min at a water-to-solids ratio of 7:1, the yield of cellulosic pulp was 70-82%. A sodium hydroxide extraction yielded a 60% cellulosic pulp and a hemicellulosic fraction available for fermentation to ethanol. The cellulosic pulps were subjected to cellulase hydrolysis at 55°C for production of sugars to support a 6-C fermentation. Hemicellulose was recovered from the liquor filtrates by acid/alcohol precipitation followed by acid hydrolysis to xylose for fermentation. Subsequent experiments have involved the fermentation of cellulosic and hemicellulosic hydrolysates to ethanol. Apparently these fermentations were inhibited by substances introduced by thermal and alkali treatment of the straws, because ethanol efficiencies of only 40-60% were achieved. Xylose from hydrolysis of wheat straw pentosans supported an ethanol fermentation by Pachysolen tannophilus strain NRRL 2460. This unusual yeast is capable of producing ethanol from both glucose and xylose. Ethanol yields were not maximal due to deleterious substances in the WS hydrolysates.

While the literature contains several methods for preparing cellulodextrins, all the methods involve several steps or involve the removal or neutralization of strong acids. A simple method has been developed using trifluoroacetic acid (TFA)-water for the hydrolysis.

Cellulose is dissolved in TFA (3 days) at room temperature. Water is added and the mixture is heated to 75°C (1 day). The TFA is removed by evaporation for reuse. The cellulodextrin mixture is diluted with water, filtered to remove any insolubles, neutralized and decolorized with an anion exchange resin and evaporated to a syrup (95% conversion). Analysis by TLC and HPLC shows a series of cellulodextrins (DP1-6) which are completely converted to ethanol by Candida wickerhamii in 4 to 5 days.

- b. Investigate selected yeasts/fungi capable of hydrolyzing cellulodextrins and starch to ethanol.

Progress: From an initial screen of 22 yeasts for their capacity to ferment cellobiose (CB), the two which fermented CB most efficiently were Candida lusitaniae and C. wickerhamii. In the test media used, C. lusitaniae produced 44 g/l ethanol from 90 g/l CB in 5-7 days. When higher CB concentration were used, fermentation ceased when the ethanol level reached 45-60 g/l. C. lusitaniae produced barely detectable levels of B-glucosidase under all test conditions. In the test media used, C. wickerhamii produced a maximum of 20 g/l ethanol. However, if the media composition is altered, ethanol yields of 45 g/l from 90 g/l CB were achieved. C. wickerhamii produced 3-5 IU/ml B-glucosidase when grown on either CB or glucose. This yeast appeared to "overproduce" the enzymes as

glucose could be detected in the CB fermentation beers as early as 24 hr. When either organism was employed in the simultaneous saccharification/fermentation of avicel, using either Trichoderma reesei or P. funiculosum cellulase as the saccharifying agent, these organisms produced from 10-30% more ethanol than did S. cerevisiae.

These organisms were also used in the simultaneous saccharification fermentation of wheat straw which had been pretreated with NaOH-H₂O₂. In all experiments, C. wickerhamii produced either an equal amount or slightly more (10-15%) ethanol than did S. cerevisiae.

Cellobiose fermentation to ethanol by calcium-alginate-immobilized Candida wickerhamii NRRL Y-2563 was investigated both in static repeated-batch and continuous-flow fermentation systems. In the repeated batch fermentations, 25-30% of the cellobiose (1, 3, 5, and 9% concentrations) was converted to ethanol within 24 hr on each of days 2 through 8, 50-65% of the cellobiose was converted. The conversion decreased to 7% on day 9 and to 0.1% by day 10. In the continuous-flow fermentation, 90-95% of the high (9%) cellobiose was converted to ethanol on the first 2 days and then gradually decreased to 25% conversion on day 5. The 25% conversion of the high cellobiose was maintained from day 5 to day 14. Ethanol production from the other cellobiose solutions (1, 3, 5%) was significantly higher for longer durations than the 9% solution but followed a similar pattern.

- c. Application of specific genetic technologies for improvement of alcohol production from plant polysaccharides by various fungi and bacteria.

Progress: Five alcohol tolerant derivatives of P. tannophilus NRRL Y-2460 have been isolated. Alcohol tolerance increased from 2.4%-2.6% in the parent to 4-6% in the derivatives. In 9% xylose fermentations, the parent culture failed to utilize 3% of the xylose after alcohol productions reached 2.2%. All of the derivatives utilized all of the xylose, with 4 of the 5 producing slightly higher levels of alcohol than the parent. In 12% xylose fermentations, the parent failed to utilize 5% of the xylose while 4 of the 5 utilized all of the xylose and produced more ethanol than the parent.

UV mutagenesis produced amino acid and modified carbohydrate utilizing auxotrophs, including, adenine, threonine, lysine, methionine and arginine deficient strains. Modifications in carbohydrate utilization included 2 glycerol negatives, 1 galactose negative, one slow xylose utilizer, one leaky mutant that utilized mannose slowly and one isolate that grew normally on glycerol but very slowly on galactose, mannose, glucose, and xylose. 6% xylose fermentations by the 2 glycerol negatives and 1 galactose negative

produced similar amounts of ethanol compared to the control. These mutants are being held in stock for use as possible genetic markers in later breeding experiments.

Diploid cells can be isolated from conjugating zygotes by micromanipulation and grown on a rich medium to prevent sporulation. Sporulation occurs by aging or transferring to a medium that allows only restricted growth. Spores from mature asci were dissected and grown as isolated stocks. When young rapidly growing cells from germinated spores are transferred to sporulation medium, sporulation does not occur as in the diploid cells. Only after conjugation and zygote formulation does sporulation arise. Evidently cells from newly germinated spores are haploid. As the population ages, mixed mating types appear to arise that then conjugate and form zygotes.

Spore isolates whose progeny showed lower degrees of self conjugation were selected and are being intercrossed and sporulated. Their progeny are being examined and stocks showing reduced frequencies of conjugation are being selected in an effort to reduce homothallism and increase heterothallic tendencies.

- d. Evaluate the production of cellulases and xylanases by Trichoderma sp. and Penicillium funiculosum.

Progress: Penicillium funiculosum NRRL 13033 has been studied for the production of B-glucosidase and xylanolytic enzymes. The highest yield of fungal protein was obtained after 7 days incubation, with maximal activities of B-glycosidase and xylanolytic enzymes was exhibited in 11 days. The optimum pH for maximal production of these enzymes was between 6.3-7.0. The freeze-dried liquor from auto-hydrolysis of wheat straw (5.0 g/l), and crude wheat straw (10.0 g/l) appeared to be the most suitable carbon source for the production of these enzymes; while NH_4HPO_4 (6.0 g/l) yielded the highest levels of B-xylosidase and B-glucosidase.

- e. Investigate biological modification of lignocellulosic residues and cellulase synthesis in a solid substrate fermentor.

Progress: Cellulase has been produced by Spicellum roseum NRRL 6371 in both wheat bran solid substrate and in liquid cultures. Growth occurs at temperatures up to and including 35°C while maximum cellulase production occurs at about 30°C. Spore suspensions have been prepared for Trichoderma reesei NRRL 3652 (QM6a), 6156 (QM4414) and 11236 (MCG77) as well as S. roseum in order to provide a uniform inoculum source for future studies.

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3. Innovative Fermentation Technology for Alcohol Production (R. J. Bothast)

- a. To increase the efficiency of alcohol production via immobilized microbial cell technologies.

Progress: Glucose was converted to ethanol by calcium-alginate entrapped Saccharomycetes cerevisiae NRRL Y-2034 cells that were 24, 48, 72, and 96-hr-old in continuous-flow and static repeated-batch fermentors. In general, older cells were more efficient than younger ones. In most cases, the continuous fermentations were better than the static ones in producing maximum ethanol yields

(5.11 g/10 g of glucose) over extended time periods. The best static fermentation (with 24 hr old cells) converted 100% of the glucose to ethanol for about 12 days, whereas the best continuous fermentation (with 96 hr old cells) converted 100% of the glucose for about 3 months.

- b. Design a continuous fermentation process which efficiently utilizes both D-glucose and D-xylose for ethanol production.

Progress: When Pachysolen tannophilus was grown in batch culture containing both D-xylose and D-glucose, it fermented all of the glucose before taking up xylose. Compared to other yeasts, P. tannophilus produced ethanol slowly and in poor yield from glucose. From this standpoint, it was deemed advantageous to carry out xylose and glucose conversions in separate stages.

A two stage continuous culture was fed a buffered solution containing 5% xylose and from 1.5 to 10% glucose. In the first stage, glucose was fermented by calcium alginate immobilized S. cerevisiae NRRL Y-2235, a yeast tested and found to ferment glucose efficiently without wastefully assimilating xylose present. The second stage, fed by the effluent of the first, contained immobilized Pachysolen tannophilus to ferment the remaining xylose. Based on the production rates of the two yeast populations, the holding times in the two stages were set at 10 and 20 hr, respectively. Reasonably efficient conversion was accomplished in both stages, nearly 100% of theoretical yield in the first and 70% in the second, provided the feed glucose concentration was below 5% and consequent first-stage ethanol concentration, below 2.5%. If these concentrations were exceeded, ethanol production in the second stage was inhibited.

- c. Investigate the effect of calcium alginate bead size on ethanol production and D-xylose consumption rates in continuous immobilized cell cultures of Pachysolen tannophilus.

Progress: When the bead radius exceeded 0.15 cm, the fermentation rate became limited by diffusion processes, rather than metabolic processes. Zero order kinetics represented fermentation in calcium alginate beads more accurately than did Monod kinetics according to data collected. Based on zero order kinetics and dependence of specific fermentation rate on bead radius, the effective diffusivity of D-xylose in the immobilized cell beads was calculated to be $6.6 \times 10^{-7} \text{ cm}^2/\text{sec}$.

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4. Energy-Saving Methods for Recovery of Usable Protein from Alcohol or Methane Fermentation Media (J. S. Wall)

- a. Compositions of residues from fermentations of corn grits, hominy feeds and degerminator meals will be established.

Progress: Corn grits, hominy feeds, and degerminator meals were fermented by a system similar to that of corn. The residue after fermentation was fractionated into distillers' grains, centrifuged solids, and solubles. Corn grits distillers' grains had much higher protein contents (65-70%) and lower fat content (2.7-3.0%) whereas corn hominy feeds distillers' grains had lower protein contents (22%) compared with corn distillers' grains. Corn

germinator meal distillers grains had similar protein and fat contents compared with distillers' grains.

- b. Investigate reverse osmosis as an energy saving method to concentrate the soluble fraction of stillage from alcohol fermentation.

Progress: Corn stillage was screened and centrifuged to remove solids, and the soluble fraction of stillage was fed into a reverse osmosis system. Reverse osmosis of a soluble fraction of corn stillage with 1.1% solids yielded 81% of initial volume as permeate. The permeate had better quality than tap water, based on conductivity. Recycled corn stillage solubles with higher solids and higher ash contents can also be processed with reverse osmosis or with ultrafiltration followed by reverse osmosis to reduce the solid and ash content of the permeate. Since no evaporation of water is involved in reverse osmosis, energy consumption is much lower than in concentration by heating.

- c. Investigate the feasibility of corn distillers' dried grains with solubles (CDGS), corn distillers' dried grains (CDG), fractions of CDGS and CDG, corn gluten meal and corn protein meal as component in blended food or other food uses.

Progress: The proximate and amino acid compositions of CDGS, CDG, and corn protein concentrate (CPC) were determined. CDG and CDGS were high in fiber (14%) and protein (25-29%) while CPC had higher protein content (48.3%) and low fiber (2.0%). All components were low in lysine but the CPC was lowest (2.0%). The products were analyzed for peroxides, free fatty acids, and available lysine before and after 8 weeks storage at 49°C. CPC showed considerable loss in available lysine due to presence of sugars. Taste panel evaluations scored CDGS too low for use in blended foods. The compositional analyses were used to calculate by computer formulations of blended foods containing CDG or CPC and milk solids, soybean meal and corn meal with compositions close to recommended standards for children's use. Twelve different formulations differing in soya protein, milk solids, and corn were prepared, analyzed, and fed to rats. PER (adjusted to 2.5 for casein) for a blend with 5% milk solids, 10% CDG was 2.22 but the fiber level was excessive, 2.2%. A blend containing 7% CDG may be acceptable depending on further taste and stability tests.

- d. Investigate additional variables to simplify processes involved in recycling distillers' solubles.

Progress: The use of a heated pot for distilling alcohol instead of injected steam reduced the volume of distillers' solubles remaining after the unsolubles are filtered off or centrifuged down from the stillage. The reduced volume resulted in an accelerated

rate of increase during recycling in the solids content of the distiller's solubles since all of the solubles could be recycled. The higher media solids content did not inhibit alcohol production after 7 recyclings.

Glycerol production was relatively constant throughout successive recycle fermentations. Typically, 4% of the sugar fermented was converted to glycerol in each fermentation. However, the glycerol content in the beer of each successive fermentation increased 0.4%, ultimately reaching 2.1% in the beer of the 5th recycle, and then declined 0.4% over each subsequent fermentation. Interaction between glycerol and soluble solids may cause a decline in glycerol and a leveling off of soluble solids after several recycles. Recovery of 2% or more glycerol in a beer containing 9-10% ethanol may be feasible.

- e. Explore composition of distillery residues from fermentation of sugar crops with view to improving their feed value.

Progress: Sugar beets were obtained from USDA, Fargo, ND, and mascerated with a Waring blender. The ground material remained particulate, each particle resembling a gel like structure entrapping the juice. Fermentation of this mixture without adding excessive water was not feasible. Efforts to use commercial pectinases to convert this mass into a liquid by breaking down the gel structure were ineffective due to the low water mobility in the mass as commercial fruit juicer was tested for removing juice from sliced beets. The beets contained about 80% water, 15% sucrose and 5% other solids. The juicer removed about 70% of the weight of the beet as juice which could be directly fermented. The juicer operated on a principal of centrifugal separation of pulp from juice.

Publications:

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WU, Y. V., K. R. SEXSON, and J. S. WALL. Reverse Osmosis of Soluble Fraction of Corn Stillage as an Energy-Saving Alternative to Drying. Cereal Chem., in press.

Other Reports:

BOOKWALTER, G. N., W. F. KWOLEK, J. S. WALL, K. A. WARNER, and Y. V. WU. Progress Report to the Congress I - Investigations on the Use of Distillers' Grains or Fractions Thereof in Blended Foods for the Foods for Peace Program and Other Food Applications, March 15, 1982.

BOOKWALTER, G. N., W. F. KWOLEK, J. S. WALL, K. A. WARNER, Y. V. WU, and M. R. GUMBMANN. Progress Report to the Congress II - Investigations on the Use of Distillers' Grains or Fractions Thereof in Blended Foods for the Foods for Peace Program and Other Food Applications. September 2, 1982.

WU, Y. V. Reverse Osmosis of Soluble Fraction of Corn Stillage as an Energy-Saving Alternative to Drying. Presented at the American Association of Cereal Chemists 67th Annual Meeting, San Antonio, Texas, October 24-28, 1982.

5. Physical/Chemical Modification of Vegetable Oils for Diesel Fuel (E. H. Pryde)

- a. Prepare methyl, ethyl, and butyl esters of soybean and other vegetable oils by transesterification for evaluation as diesel fuels.

Progress: Fatty esters obtained from vegetable oils by transesterification have promising potential as alternative diesel oil fuels because of their improved viscosity and volatility properties compared to triglycerides. Efforts have been directed toward determining which reaction variables affect the yield and purity of these esters. The ester content of methyl, ethyl, and butyl esters from transesterified sunflower oil was strongly influenced by the molar ratio of alcohol/vegetable oil, the type of catalyst (basic or acidic), and reaction temperature. At a 6:1 molar ratio of alcohol/sunflower oil, 0.5% sodium methoxide catalyst, and reaction temperatures near the boiling points of the alcohols, the weight percent of the three esters ranged from 96-98 after 1 hr. At the stoichiometric ratio of 3:1, the range decreased to 81-88% and continued to decrease at lower ratios. Transesterification with acid catalysis was much slower than with basic catalysis. With 1% sulfuric acid and a 30:1 molar ratio of alcohol/soybean oil, the methyl, ethyl, and butyl esters required 69, 21, and 3 hr, respectively, to be formed at ca. 94% when the reactions were conducted near the various boiling points of the alcohols (65-117°C). When all three alcohols were allowed to react at 65°C for 45 hr, the weight percent range of the esters decreased to 73-87. At moderate temperatures (32°C), vegetable oils can be 99% transesterified in about 4 hr with alkaline catalyst.

- b. Compare the transesterification of crude vs. refined soybean, sunflower, peanut, and cottonseed oils.

Progress: All four refined oils responded about the same to decreasing molar ratios of methanol/vegetable oil. At a 6:1 molar ratio, ester content ranged from 93-98%, and decreased to 74-84% at a 3:1 molar ratio. Crude oils were also transesterified at a 6:1 molar ratio. The percent ester yields (and % purity) from crude cottonseed, soybean, sunflower, and peanut oils were 86(98), 86(96), 85(95), and 69(96), respectively. The reduced yields from the crude oils compared to the refined oils are due to gums and other extraneous materials present in the crude oils.

- c. Determine the effect of the molar ratio of alcohol/vegetable oil on glycerol yields.

Progress: The recovery of glycerol formed during the transesterification of refined sunflower, peanut, and cottonseed oils has been determined. Glycerol yields were about the same for all three oils, and depended upon the molar ratio of alcohol/oil employed. With sunflower, for example, the molar ratios and resulting yields of glycerol were: 6:1, 100%; 5:1, 100%; 4:1, 79%; 3:1, 60%; 2:1, 17%; 1:1, 0%.

- d. Improve the Lactoscan quantitative analysis of transesterification reaction products by extending the concentration range and types of compositions which can be analyzed; optimize analytical procedures to improve sensitivity, resolution, and increase output.

Progress: The concentration range of methyl esters and triglycerides which can be analyzed has been extended from 95-50% to 97-1% for methyl esters, and from 30-3% to 97-1% for triglycerides. Diglycerides and monoglycerides can now be analyzed at 40-1% and 20-1%, respectively. Response factors for ethyl and butyl esters have been determined. Screening of a number of solvent systems for the TLC phase has pinpointed one system which optimizes resolution. Changes in instrument and method parameters has also improved sensitivity and resolution. A procedure has been developed whereby 30 samples can be analyzed in 2-3 hr.

- e. Assist in technology transfer and development of basic information for the use of vegetable oils as emergency alternative fuels in crop production.

Progress: A 1-day symposium on Biomass Energy was organized for the 16th Great Lakes Regional Meeting, American Chemical Society, at Illinois State University in June. Assistance was provided to the Program Committee for the International Conference on Plant and Vegetable Oils as Fuels, August 1982, and to the development of plans for a future international conference to be held in 1985.

Papers from the symposium on the use of vegetable products as diesel fuel at the American Oil Chemists' Society will be published as a unit in the Society Journal. Another symposium on the same topic is being organized for the May 1983 meeting of the Society in Chicago.

Publications:

FREEDMAN, B. and E. H. PRYDE. Fatty Esters from Soybean Oil for Use as a Diesel Fuel. "Vegetable Oil as Diesel Fuel" Seminar II, October 21-22, 1981, NAEC, Peoria, Illinois.

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REED, D. K., B. FREEDMAN, and T. L. LADD, JR. Insecticidal and Antifeedant Activity of Neriifolin Against Codling Moth, Striped Cucumber Beetle, and Japanese Beetle. J. Econ. Entomol. (Accepted March 22, 1982.)

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Other Reports:

BAGBY, M. O. Vegetable Oils for Diesel Fuel. Presented at 24th Annual Meeting American Bakers' Association Technical Liaison Committee with USDA, Albany, CA, February 1982.

PRYDE, E. H. Chemicals and Fuel from Commercial Oilseed Crops. Presented at American Association for Advancement of Science, Washington, D.C., January 3-8, 1982.

PRYDE, E. H. Vegetable Oils for Diesel Fuels. Presented at Central Illinois Section, American Society of Lubrication Engineers, Peoria, Illinois, January 11, 1982.

PRYDE, E. H. Vegetable Oils as Diesel Fuels. Presented at the Spring Conference of the Illinois College Automotive Instructor Association at Illinois Central College, East Peoria, Illinois, April 22, 1982.

PRYDE, E. H. Vegetable Oil Fuels for the U.S. and Foreign Countries. Presented at the Illinois Agricultural Program Review on alternate fuels from agricultural products--Where Do We Go From Here?, Bloomington, Illinois, April 26, 1982.

PRYDE, E. H. Vegetable Oil Fuel Standards. Presented at the American Society of Agricultural Engineers International Conference on Plant and Vegetable Oils as Fuels, Fargo, North Dakota, August 2-4, 1982.

PRYDE, E. H. Renewable Resources - Fuels from Alternative Chemicals. Presented at Illinois Soybean Program Operating Board Update on Research at NRRC, Peoria, Illinois, August 18, 1982.

6. Long-Term and Endurance Engine Tests with Vegetable Oil Products as Diesel Fuel (Cooperative Agreement - North Dakota State University)

- a. Specific Objective: Determine the technical feasibility of vegetable oil products as fuel in diesel engines used for crop production.

Progress: Following the recommended Engine Manufacturers' Association for the 200-hr engine test cycle, the Engine Test Laboratory of Allis-Chalmers at Harvey, Illinois, has evaluated several experimental fuels proposed for use in direct injection diesel engines, after first establishing a data base line with Phillips reference No. 2 diesel oil.

25/75 Blend. This blend of 25% alkali-refined sunflower oil in 75% No. 2 diesel oil did not pass the criteria established for the EMA cycle. After 73 hr of operation, there was a 5% drop in power and significant differences in fuel injection line pressure traces were experienced. Injection nozzles were changed at that point. After 170 hr, the second set of nozzles showed abnormal carbon build-up at the tips, a reduction in orifice diameter, and excessive wear and sticking. There were heavy black deposits on all four exhaust valve stems. The carbon build-up on all parts showed no tendency to break off. There was no problem with the lubricating oil.

25/75 Blend with Additives. A barium smoke-suppressant additive (Lubrizol 565) was added to the 25/75 blend at several concentrations (0.125, 0.25, and 0.5%). After 30 hr of engine operation, there occurred a considerable reduction in power output and an increase in smoke at all levels. Excessive, irregular deposits were formed around the nozzle tips. No reduction in carbon build-up was observed in any part of the engine. The injector nozzle orifices remained clean, but there was increased

deposit accumulation in the combustion chamber and on the tips compared to the run without additives. There was no problem with the lubricating oil.

Because of the poor results, a short control run with the 25/75 blend was made in the absence of additives. This control run showed considerably less deterioration in performance compared to the one with the smoke suppressant additive. It was concluded that the barium smoke suppressant additive did not aid in the combustion of sunflower oil, as it does with diesel oil.

A short run of 60 hr was made with the 25/75 blend with an ashless detergent additive (Lubrizol 552) present in the fuel at a concentration of 0.03%. The engine showed stable performance, and there was normal build-up of carbon on the nozzle tips, top of the pistons and cylinder head. This additive also kept the inside of the nozzles clean. Lubricating oil performance was satisfactory.

Microemulsion. A nonionic microemulsion consisting of sunflower oil (8 parts), 95% ethanol (2 parts), and 1-butanol (5 parts by volume) gave mixed results. Favorable results include adequate engine performance throughout the 200 hr, shorter spray penetration (spray did not hit cylinder liners) than with the 25/75 blend, and no significant build-up of carbon in or around the injector nozzles. Unfavorable results included: sporadic needle sticking, inability to start cold engine without starting aids, significant dilution and thickening of the lubricating oil, and incomplete combustion during start and low idle.

It was concluded that reduction in fuel viscosity as in the microemulsion was not the only factor, although an important one, in obtaining improvement in vegetable oil performance in a direct-injection diesel engine. Another factor may be the degree of unsaturation in the vegetable oil.

Sunflower Methyl Esters. This test is now in progress.

Publications:

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KAUFMAN, K. R. Testing of Vegetable Oils in Diesel Engines. In "Fuels and Chemicals for Oilseeds: Technology and Policy Options," E. B. Shultz, Jr. and R. P. Morgan, eds. AAAS Selected Symposium/Westview Press, Washington, D.C., accepted for publication, Chapter 9.

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KAUFMAN, K. R. Use of Vegetable Oils as an Alternative Fuel. Lecture presented at the International Combustion Engineers Class, Mechanical Engineering 462, College of Engineering, North Dakota State University, Fargo, North Dakota, February 16, 1982.

KAUFMAN, K. R. Vegetable Oils as a Substitute for Diesel Fuel. Lecture presented at the University of Alberta, Edmonton, Alberta, April 27, 1982.

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KAUFMAN, K. R. Using Your Crop as a Fuel. American Soybean Association Exposition, Minneapolis, Minnesota, August 6-9, 1982.

KAUFMAN, K. R. The Use of Vegetable Oils for Diesel Fuel. Public statement given before the U.S. Department of Energy Advisory Committee on Federal Assistance for Alternative Fuels Demonstration Facilities, Bismark, North Dakota, September 29, 1982.

KAUFMAN, K. R. Overview of the Potential for Vegetable Oil as a Diesel Fuel, Diesel Engine Testing of Vegetable Oil Fuels, and Extraction, Processing, and Economics of Vegetable Oil Fuels. Three lectures presented at the Texas A & M University, College Station, Texas, November 17-18, 1982.

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ZIEJEWSKI, M. Laboratory Endurance Test of a Sunflower Oil Blend in a Diesel Engine. Presented at the International Conference on Plant and Vegetable Oils for Fuel, American Society of Agricultural Engineers, Fargo, North Dakota, August 2-4, 1982.

7. Model for Determining Feasibility of Potential Energy Crops (Cooperative Agreement - Purdue University)

- a. Specific Objective: Establish critical factors that influence energy inputs and outputs for potential energy crops, and identify energy-efficient crop and process systems for production of liquid fuels.

Progress:

Computer model subprograms are being developed for evaluating potential energy crops such as sweet sorghum, sugar beets, potatoes, and Jerusalem artichoke. A time-incorporated linear program named Purdue Crop Budget Model B-10 was used to examine sweet sorghum as a crop alternative for a typical Midwest corn farm. Preliminary observations show that the most profitable farm plan included a mix of full-season corn and sweet sorghum. The principal advantage was provided by more efficient use of field machinery allowed by extended planting and harvesting periods. The input data for sorghum production were artificial. To provide production input data for future model testing, sweet sorghum was planted in forty 4-row plots at the Purdue University Agronomy Farm. Half of the plots were planted June 7 and the remainder July 6. Harvesting of

the 2 center rows was conducted at 4 stages: half bloom, soft dough, maturity, and post maturity. The NAEC sorghum uniform production test protocol is being followed to provide appropriate data.

Publication:

BENDER, D. A., R. M. PEART, and M. O. BAGBY. Systems Dynamics of Energy Crops. Proceedings, 1982 Summer Meeting American Society of Agricultural Engineers, University of Wisconsin, Madison, Wisconsin, June 27-30, 1982. Paper No. 82-3089.

8. Production Problems of the Oilseed Crop, Crambe (*Crambe abyssinica*) (Cooperative Agreement - Murray State University)
 - a. Specific Objective: Renew and increase the supply of viable crambe abyssinica seed in ARS stocks.

Progress: Plans have been made for seed renewal and increase plantings (30 acres) in March-April, 1983.

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